



स्वास्थ्य दर्शन





**PROCEEDINGS AND PAPERS**  
**OF THE**  
**FIRST INTERNATIONAL**  
**CONGRESS OF SOIL SCIENCE**

**JUNE 13-22, 1927**  
**WASHINGTON, D. C., U. S. A.**

**COMMISSION III**  
**AND**  
**COMMISSION IV**

**Edited under the supervision of the Executive**  
**Committee of the American Organizing Committee**

**By R. B. Deemer, assisted by**  
**P. R. Dawson and A. R. Merz**

**PUBLISHED BY**  
**THE AMERICAN ORGANIZING COMMITTEE**  
**OF THE**  
**FIRST INTERNATIONAL CONGRESS OF SOIL SCIENCE**  
**WASHINGTON, D. C.**  
**1928**

These Proceedings may be purchased from the Executive Secretary  
of the First International Congress of Soil Science, United States  
Department of Agriculture, Washington, D. C., U. S. A.

Price per Set to non-members \$10.00

## AMERICAN ORGANIZING COMMITTEE

Milton Whitney, *Honorary Chairman*

### EXECUTIVE COMMITTEE

Oswald Schreiner, *Chairman*

J. G. Lipman

C. F. Marbut

K. F. Kellerman

A. G. McCall, *Executive Secretary*

### REGIONAL REPRESENTATIVES

Alabama, M. S. Funchess  
Arizona, P. S. Burgess  
Arkansas, Martin Nelson  
California, D. R. Hoagland  
Colorado, W. P. Headden  
Connecticut, M. S. Morgan  
Delaware, T. F. Manns  
Florida, R. W. Ruprecht  
Georgia, J. R. Fain  
Idaho, G. R. McDole  
Illinois, W. L. Burlison  
Indiana, S. D. Conner  
Iowa, P. E. Brown  
Kansas, R. I. Throckmorton  
Kentucky, J. S. McHargue  
Louisiana, C. W. Edgerton  
Maine, G. E. Simmons  
Maryland, A. G. McCall  
Massachusetts, S. B. Haskell  
Michigan, M. M. McCool  
Minnesota, F. J. Alway  
Mississippi, J. R. Ricks  
Missouri, M. F. Miller  
Montana, L. F. Giesecker  
Nebraska, W. W. Burr  
Nevada, Robert Stewart  
New Hampshire, F. W. Taylor  
New Jersey, J. G. Lipman

New Mexico, H. V. Jordan  
New York, T. L. Lyon  
North Carolina, W. B. Cobb  
North Dakota, H. L. Walster  
Ohio, F. E. Bear  
Oklahoma, H. J. Harper  
Oregon, W. L. Powers  
Pennsylvania, J. W. White  
Porto Rico, H. C. Henriksen  
Rhode Island, B. L. Hartwell  
South Carolina, H. W. Barre  
South Dakota, A. N. Hume  
Tennessee, W. H. MacIntire  
Texas, G. S. Fraps  
Utah, J. E. Greaves  
Vermont, E. Van Alstine  
Virginia, W. B. Ellett  
Washington, F. J. Sievers  
West Virginia, E. P. Deatrick  
Wisconsin, E. Truog  
Wyoming, A. F. Vass  
Johns Hopkins, B. E. Livingston  
Washington, D.C., C. F. Marbut  
Washington, D.C., Oswald Schreiner  
Washington, D.C., K. F. Kellerman  
Canada, F. T. Shutt  
Canada, F. A. Wyatt  
Canada, R. Harcourt



## PREFACE

The American Organizing Committee here acknowledge with due appreciation the financial assistance rendered by the business organizations and individuals who contributed unstintedly to the fund which financed the meetings and the transcontinental tour. The hearty and earnest coöperation of the Honorable Secretary of the United States Department of Agriculture and of the American Society of Agronomy contributed to the success of the Congress and is gratefully acknowledged. Likewise their thanks are due to the United States Chamber of Commerce for the splendid facilities provided for the meetings and the exhibits of the Congress, and to the Pan American Union and the National Gallery of Art for the use of their buildings for social functions.

The papers of the several commissions of the First International Congress of Soil Science, that have been submitted for publication, are printed in the original language of the author, with the exception of those submitted in Russian, and the order of arrangement is that adopted in the Abstracts of the Proceedings. Those papers that were presented at the various sessions but were not sent in for publication are given by title at the end of their respective Commission.

The thanks of the American Organizing Committee of the International Congress of Soil Science and of the editor are due to Dr. A. R. Merz and Mr. P. R. Dawson for editing all papers written in foreign languages and to Mr. E. F. Snyder for editing many of the papers dealing with the subject of hydrogen ion investigations. They are particularly indebted to Mr. Dawson for the preparation of manuscript from material appearing in long hand. They also wish to acknowledge their indebtedness to Dr. J. S. Joffe for translating all of the papers given in Russian and to Mr. S. H. McCrory for the revision and editing of many of the papers of Commission VI.

The abbreviations used throughout the Proceedings and the Papers are those adopted by the American Chemical Society. In so far as possible references are given in a list at the end of each paper and are referred to by numbers in parentheses in the text. The volume number is given first and then the first page reference of the periodical. Otherwise the reference is given as submitted by the author. The summaries of papers have been omitted, since they are to be found in the abstracts distributed to members during the meetings of the Congress at Washington.

R. B. DEEMER,  
*Editor*



# CONTENTS

Contents for Commission IV, page 379

	PAGE
Winogradsky, S., The direct method in soil microbiology and its application to the study of nitrogen-fixation . . . . .	1
Rossi, G., and Riccardo, S., The direct microscopical and bacteriological examination of agricultural soil . . . . .	9
Wilson, J. K., The number of ammonia-oxidizing organisms in soils . . . . .	14
Lochhead, A. G., The advisability of standardizing the methods used for the quantitative determination of soil bacteria, and the changes produced by them . . . . .	23
Bristol-Roach, B. M., The present position of our knowledge of the distribution and functions of algae in the soil . . . . .	30
Thom, C., Present and future studies of soil fungi . . . . .	39
Brierley, W. B., Jewson, S. T., and Brierley, M., The quantitative study of soil fungi . . . . .	48
Magrou, J., Les champignons de Mycorhizes et leur rôle dans le développement des plantes . . . . .	72
Conn, H. J., Bacterial population of soil . . . . .	92
'Sigmond, A. A. J. de, Telegdy-Kováts, L., and Zucker, F., The effect and importance of the absorbing complex (Humus-Zeolite) in soils as regards soil bacteria . . . . .	96
Brown, P. E., and Benton, T. H., Microorganisms in some soil profiles in Iowa . . . . .	100
Gainey, P. E., The occurrence of <i>Azotobacter</i> in soil . . . . .	107
Brenner, W., Über Stickstoffbindung durch frei lebende Mikroorganismen im Boden . . . . .	118
Stapp, C., Die Stickstoffbindung durch Bakterien . . . . .	125
Cunningham, A., and Jenkins, H., The coccoid phase of <i>Bacillus amylobacter</i> , A. M. and Bredemann . . . . .	144
Asō, K., and Yoshida, R., The application of the serum-reaction in the classification of <i>Azotobacter</i> . . . . .	150
Gorini, C., Sur la manière de se comporter de l' <i>Azotobacter chroococcum</i> dans le lait . . . . .	152
Hirai, K., Influence of thorium and uranium salts on nitrogen-fixation by <i>Azotobacter</i> . . . . .	154
Hirai, K., and Hino, I., Influence of soil protozoa on nitrogen-fixation by <i>Azotobacter</i> . . . . .	160
Erdmann, L. W., and Fife, J. M., Studies on nitrogen-fixation by inoculated soybeans . . . . .	166
Ohkawara, S., The influence of nitrates and sulfates on the nodule bacteria and nodule formation of Genge, Lupin and Serradella . . . . .	172
Asō, K., and Ohkawara, S., Studies of the nodule bacteria of Genge . . . . .	183
Richmond, T. E., Some effects of stock and scion relationship upon the legume nodule organism . . . . .	185



Bal, D. V., A common error in the method of total nitrogen estimation in soils and its bearing on the results of nitrogen-fixation experiments . . . . .	190
Niklewski, B., The transformations of nitrogen in manure . . . . .	196
Barthel, C., and Bengtsson, N., Nitrogen availability in fungus and bacterial cells for nitrification and cellulose decomposition in the soil . . . . .	204
Waksman, S. A., and Tenney, F. G., Nitrogen transformation in the decomposition of natural organic materials at different stages of growth . . . . .	209
Greaves, J. E., The influence of soluble salts and organic manures in soil nitrogen . .	213
Greaves, J. E., Soil microbial stimulants . . . . .	222
Hendrick, J., Some notes on the conditions of nitrification . . . . .	229
Runow, E., and Michoustine, E., Über die Bildung von Nitriten durch Bacterien . .	237
Beaumont, A. B., Nitrification in Massachusetts soils . . . . .	240
Michoustine, E., L'Ecologie des bactéries du sol, qui produisent la fermentation de l'Urée . . . . .	250
Schreiner, O., and Dawson, P. R., The chemistry of humus formation . . . . .	255
Shorey, E. C., Non-humus constituents of the humus extract . . . . .	264
Itano, A., The carbon-nitrogen ratio and microbiological investigations of the soil in rice fields . . . . .	269
Guittonneau, G., L'oxydation microbienne du soufre dans ses rapports avec l'évolution de la matière azote dans le sol . . . . .	274
Lieske, R., Der gegenwärtige Stand unserer Kenntnisse von den Eisenbakterien . .	285
Starkey, R. L., and Halvorson, H. O., The rôle of microorganisms in transformations of iron in nature . . . . .	290
Roudakov, K. T., La réduction par voie biologique des phosphates minéraux dans le sol . . . . .	296
Clark, N. A., Purification of chemicals for use in the preparation of nutrient solutions. I. The crystallization of phosphoric acid . . . . .	300
Lemmaermann, O., Untersuchungen über die Bedeutung der Bodenatmung für die Kohlensäureernährung der Kulturpflanzen . . . . .	305
Lipman, J. G., and Blair, A. W., Microbiological aspects of green manuring . . . .	312
Rayner, M. C., The rôle of Mycorrhiza in plant nutrition . . . . .	317
Bonazzi, A., Some consideration on methods of soil biology . . . . .	325
Zolcinski, J., The new genetic physico-chemical theory about the formation of humus, peat and coal. The rôle and significance of biological factors in these processes . . . . .	335
Bonazzi, A., Plant residues in tropical soils. I. Sugar cane trash . . . . .	339
Ciferri, E., Relación entre las levaduras y la población microorganica del suelo . .	350
Steiner, G., The nemic population of the soil . . . . .	360
Vernander, N. B., and Sokolovskii, A. N., The study of humus: chernozem humus as a polydisperse system . . . . .	367

---

## PROFESSOR V. L. OMELIANSKI

Just as this volume went to press, news came from Russia that Professor V. L. Omelianski, member of the Russian Academy of Sciences and noted bacteriologist, newly elected president of the Third Commission of the International Society of Soil Science, died (on April 21, 1928) after a very brief illness, in one of the Caucasian mountain resorts.

Russian Science has been suffering lately considerable losses through the death of some of its outstanding representatives. Only within a period of six or seven months, three men who have made notable contributions to Soil Science have died and the only three who have held official positions in the International Society of Soil Science. At first Professor Glinka, then Professor Omelianski, and soon after Professor Neustruev; all have died largely as a result of severe privations that they had to experience during the revolutionary and hunger periods in Russia; their health was then severely broken down and now, even when material conditions have improved quite considerably, the consequences become evident.

In the death of Professor Omelianski, bacteriology in general and Russian bacteriology in particular, have lost one of the most outstanding workers. His contributions to the study of cellulose decomposition and of nitrogen-fixing bacteria and his associations with Professor Winogradsky have placed him in the front ranks of bacteriologists. The general texts of bacteriology, the scientific monographs written and the journals edited will remain, however, his outstanding contributions. Unfortunately these have been very little known outside of Russia. His textbook on bacteriology has gone through six editions within a short period of time.

Born in 1867 in the Ukraine, Omelianski was educated in the Gymnasium of Zhitomir and, after graduation in 1885, he entered the University of St. Petersburg, where he specialized in chemistry. In 1893 he was invited by Professor Winogradsky to enter the Division of General Microbiology of the Institute of Experimental Medicine in St. Petersburg as chemical assistant. During the twenty-year period, between 1893 and 1912, Omelianski made his most outstanding contributions to the subject of bacteriology, largely in collaboration with Professor Winogradsky. Since 1913 he was made the Head of the Division of General Microbiology. He was at the same time lecturer on microbiology in the Higher Institute for Women in St. Petersburg. In 1915 he was made Doctor of Botany. In 1920 he was elected as corresponding member of the Academy of Science and in 1924 he was made active member of the Russian Academy of Science. Since 1918 he was also given charge of the investigations on



PROF. V. L. OMELIANSKI, 1867-1928

general microbiology at the Lesgaft Institute and later of the Institute of Experimental Agronomy. He was editor of the Archives of Experimental Medicine and of a series of volumes on the Progress of Biological Sciences. During 1914 to 1920, there appeared from his laboratory a series of studies on the morphology and physiology of the nitrogen fixing-bacteria *Clostridium pasteurianum* and of *Azotobacter*. These studies were culminated by the appearance in 1923 of a monograph on the "Fixation of atmospheric nitrogen by soil microorganisms."

Omelianski came from the Russian middle class, his father being teacher of ancient languages at the Gymnasium of Zhitomir and later director of that Gymnasium. Those that have known him intimately found in him a very sincere advisor and best friend.

SELMAN A. WAKSMAN



## **COMMISSION III**



# THE DIRECT METHOD IN SOIL MICROBIOLOGY AND ITS APPLICATION TO THE STUDY OF NITROGEN-FIXATION

S. WINOGRADSKY

*Pasteur Institute, Paris, France*

## INTRODUCTION

The question as to whether we are justified in speaking of Soil Microbiology as of an existing, youngest branch, of Microbiology has repeatedly been asked and differently answered. The writer is of the opinion, already formulated in his address in Rome and elsewhere, that such a branch, if born, is yet in its infancy. What we call Soil Microbiology is no more than a chapter of General Microbiology treating of microorganisms isolated from the soil and hypothetically admitted to be taking part in some processes which are characteristic of this natural medium. Remarkable work has been done in this direction by numerous investigators in many countries, and the accumulated knowledge of thirty-five years' work must be regarded as acquired scientific knowledge. Without doubt, it forms a necessary introduction to Soil Microbiology, *but it cannot be taken for Soil Microbiology itself*. The difference between the general topics of the two is too wide for them to figure under the same heading.

In fact, the subject of the General Microbiologist is the study of the morphology and physiology of species, which have been chosen by him or which have in some way fallen into his hands, whereas the aim of the Soil Microbiologist is to study the biological agents of soil processes, *such as they are given in nature*, in their original soil and under the special conditions of that soil. The former is free to use in his experiments all means suggested by certain standard programs or by his own ideas; but the latter has to pursue his investigations, as exactly as possible, in the boundaries placed by nature itself. In short, there is in principle the same difference between the two, as between the agriculturist or horticulturist, on the one hand, and the florist or ecologist, on the other. This granted, it necessarily follows that aims so divergent cannot be served by one and the same method.

The writer will call attention to the two leading principles on which the highly elaborated methods of General Microbiology are based. The first is the obligatory pure culture method. As far as the second is concerned the plurality of conditions, under which this pure culture has to be grown, may be mentioned for the purpose of studying the reactions of the given



species, which are regarded as characteristic. It does not matter, whether these actions and reactions may be quite impossible in nature,—such as the action produced by certain poisons, adaptation to chemicals, influence of high temperature, etc.,—these experiences are eagerly pursued, inasmuch as they can lead to new variations or to industrial application, such as the production of glycerol from sugar by yeast or the formation of acetone and butyl alcohol by anaerobic bacteria.

One point still deserves consideration, since it touches more the nature of action rather than a well defined principle or method, namely, the use of laboratory collection cultures, which have been isolated or acquired a long time ago and which have gone through numerous generations by transfers. Certainly, by keeping them in culture instead of isolating them freshly from soil, one saves time and labor, but one has reason to doubt, whether these domesticated hothouse organisms can be considered to be identical with the soil species which they are believed to represent.

Now the writer would suggest to imagine a microbiologist at work on some important soil process by the use of methods developed in General Microbiology. Using the soil as an inoculum of a solution of a special composition, he tries to pass as rapidly as possible through the preliminary stage of crude or enrichment culture, and to start to isolate the organism, generally using a standardized solid medium. Conscious of the fact that only investigations with pure cultures are considered as trustworthy, the sooner he isolates these the better, for the proper study can begin only after the pure cultures have been isolated. Suppose he succeeds in reproducing in pure culture a certain amount of decomposition of some cellulose preparation, and he has satisfied himself by having isolated a cellulose destroying bacterium from the soil. Is he justified in drawing conclusions? Nothing can be less proven than this assumption. Even granted that the form isolated possesses some power of destroying some of the cellulose preparation *in pure culture*, nothing can be said concerning its specific activity *in the soil*, where it may come in contact with a physically and chemically different sample of cellulose, but chiefly where it may have to compete with a much more powerful cellulose destroying organism, which attacks rapidly all of the energy source leaving nothing to the weaker organism. The cellulose decomposing power of the latter is then doomed to be manifested only in pure culture, where it is not handicapped by competitors, and it remains in nature only as a potential cellulose decomposing organism.

The data obtained by the pure culture method are rendered all the more fallacious by the fact frequently observed, that the most powerful agents are more specific in their action, i.e. they are adapted to a much narrower range of conditions than the less specialized agents. The necessary consequence of this phenomenon is that the organisms which are of little or no importance at all in particular process in nature can be

readily isolated, while powerful agents, which are probably the sole agents in transforming the energy material, are methodically overlooked on standard media. It becomes evident that even the most extensive and the most able application of the pure culture principle can lead to no other result than a more numerous collection of forms in pure state, concerning the rôle and rate of action of which in the soil one can have only a very faint idea.

Pure cultures can give to soil biochemists only a general and somewhat vague idea of the functions of soil organisms, but not a sufficient basis for the study of the part played by them in soil processes. Especial caution must be exercised against the tendency to explain the part played by soil organisms on the basis of the phenomena observed in the study of cultures of organisms chosen by the biochemist himself and not by nature, where agents still unknown may impress upon the process quite an unexpected character.

What can be said then in regard to the second principle mentioned above, i. e. the obligatory pure culture of an organism upon a whole series of media to complete the characteristic of the form isolated? The writer cannot help thinking that these cultural manipulations,—such as trying to grow typical soil microorganisms on milk, beef broth, broth gelatin and many other “bacteriological media,” standardized or not, are quite devoid of interest to Soil Microbiology. Such study may present an interest, as indicated, for the general microbiologist trying to get an insight into the plasticity of the organism, its variability or the possibility to deviate its function,—problems that have not much to do with its “wild” state. This is especially true, since the behavior of the respective pure culture could not be attributed to the original soil species, but rather to a cultural variety issued from the former through the special influence of a new mode of existence. Is it not evident, therefore, that the soil microbiologist, having plenty to do with the study of natural phenomena, should rather avoid questions of this kind at the risk of obscuring his own task?

These considerations lead inevitably to the perhaps somewhat startling conclusion, that the value of the above two principles of General Microbiology become negative when applied to the special problems of Soil Microbiology: the procedure based on them appears as unreliable and in some sense misleading. Logically then there is no other solution than to place these methods upon a second plan, as auxiliary methods of no obligatory application to Soil Microbiology. On the contrary, principal stress must be laid on so-called crude cultures of an elective character, arranged in such a manner as to allow observation of the free play of all biological factors in a given soil. Investigations are to be carried out, of course, with “wild species obtained directly from their original soil.”

The writer was certainly not the first to criticize the current method.

Incidental critical remarks can easily be found in the work of certain leading soil scientists, as Sir John Russell, or bacteriologists, as Dr. H. J. Conn, and of a few others. Dr. Conn deserves the credit for having pointed out the importance of adding a direct microscopic method to the exclusively cultural methods used in Soil Microbiology, and for devising a method for a microscopical examination of the soil. Too imperfect for experimental researches, this first method is, nevertheless, meritorious in having attracted attention to the question of biological soil microscopy, a subject so completely neglected until very recently.

But no serious attempt was ever made to discover a general method less conventional and more adequate to soil problems than the current one, until the writer, after having briefly indicated in 1923 to 1924 the basis of his so-called *direct method*, presented it in full detail in 1925 (1).

### DESCRIPTION OF DIRECT METHOD

The general idea of this method is to keep conditions as nearly natural as possible. Consequently, no isolation, no pure cultures, no "bacteriological media" are admitted. The multiplication and activity of soil species, or groups, are studied in the original soil itself, in provoking, by addition of different substances or by physical means, the formation of so-called *natural* or *spontaneous cultures*, which are controlled by repeated microscopic examination, after a method devised by the writer. To secure the organisms in colonies, out of the soil, *silica-jelly plates* of an *elective composition* are used, upon which particles of the soil samples are sown. The earth or the silica-jelly, rendered elective by proper means, give rise to a development of specific organisms so nearly exclusive, that the action observed can be attributed to them without doubt. If they are accompanied by missmates, there are means to determine the part played by them, if any.

A study of the reactions of one or more of soil species in pure culture can, of course, be pursued if desirable, but keeping in mind the fact that the potentialities to be thus established are not directly applicable to soil processes.

Such were the principles and the *modus operandi* devised in the writer's methodological researches. Now it seemed important to him to submit the new method to an extensive experimental trial in applying it to the study of a most interesting group of soil agents. He selected the nitrogen-fixing group and decided to proceed *ab ovo*, as if nearly nothing were known about it, using thereby the new procedure exclusively and putting totally aside the old one (2).

At first the development of spontaneous cultures was brought about by adding small quantities of various energy sources to a fertile soil, which was then kept in a shallow stratum at optimum moistures and temperature, or was packed in glass cylinders, to obtain anaerobic conditions.

Rapid multiplication of characteristic big cocci ensued in the first case, whereas innumerable *Clostridium* (*Amylobacter*) forms were found under anaerobic conditions. When evaluated roughly by the direct microscopic examination, the number of cocci attained in 48 hours was found to reach about a hundred million and after 24 hours more,  $1\frac{1}{2}$  to 2 billions per gram of soil.

The exclusive predominance of the above mentioned cocci, as well as of the *Clostridium* was most striking (as seen in the photographs of the original paper).

The aerobic experiment was repeated, but, in addition to the carbonaceous matter, small quantities of nitric nitrogen were also added. The soil population presented at once quite a modified aspect. When N:C ratio was 1:100 or somewhat more, the microscopic field is found after 24 hours to be covered with numerous bacilli; though the cocci will slowly appear later, their abundance will hardly reach  $1/25$  part of the population formed without the addition of nitrogen. When the ratio N:C is raised to 25:100, none of the large cocci will be formed, their resting stages being not numerous enough to be easily found in the preparations.

These observations lead to the conclusion that available nitrogen, even in the smallest doses, has the effect of inhibiting and suppressing the development of the characteristic cocci if it were toxic to them. But of course, this is not a question of toxicity; it is a simple consequence of the fact that the rate of multiplication of the bacilli is much more rapid than that of the large cocci, so that the latter are invariably depressed, in all cases, where the ratio N:C is sufficient for the bacilli; only when this ratio is reduced too low to permit the development of the bacilli, the field is left free for the slower growing cocci to pervade the medium in consuming the energy bearing material.

Now what are these large cocci? Their apparent indifference to the presence of available nitrogen suggests their nitrogen-fixing ability; their form and size resemble cultivated forms of *Azotobacter*, and they themselves are found to be soil *Azotobacter* forms, as can be easily demonstrated by isolating them from the soil.

The question arises, however, how one could explain the difference in the influence of nitric nitrogen between the soil *azotobacter* and the cultivated form of this organism? For the latter never shows the highly characteristic negative reaction towards available nitrogen; on the contrary, the cultivated species react to available nitrogen with an extra abundant growth, as was repeatedly noted. This is a new and instructive example, how different the behavior of a species in pure culture is from a natural form: in pure culture, safe from competition, the organism can be highly favored by the addition of a certain substance, which is used readily as a nutrient, while the same substance may become in the soil quite inimical, for the evident reason that it offers greater advantages

to powerful antagonistic organisms, with which the above mentioned organism has to compete for the available energy material. Facts of this kind are important, since they throw light on the decisive part that this competition plays in regulating the biological soil processes.

Except for this mode of spontaneous culture which is controlled by microscopic examination, an easy method was devised for obtaining *macroscopic spontaneous cultures*, i.e. Azotobacter colonies on their own soil sample. Sifted earth containing 5 per cent pulverized starch is worked with a little water to a thick paste, then packed into small 5 cm. Petri dishes; the surface is polished with a glass slide moistened with water and the plates incubated at 30°; where it is present Azotobacter colonies will appear after 48 hours, rarely later, in nearly pure state, covering the surface of the soil plate more or less densely.

Passing to the plate-cultures on elective silica-jelly, Petri dishes of different dimensions are used:

(1) *Elective Silica-Jelly*.—Plates 9 to 10 cm. in diameter are inoculated with 50 or 100 smallest grains of soil deposited on the surface of the jelly; these plates are convenient for rapidly discovering the presence of Azotobacter in the soil.

(2) *Method of Large Plates*.—Large 20 cm. plates for determining the density of Azotobacter cells in a given sample of soil and at the same time its "power of nitrogen-fixation;" the plates are inoculated with one gram of soil, on a dry basis, incubated the necessary length of time, then dried and digested by the Kjeldahl method.

The Azotobacter colonies appear after 48 hours' incubation, followed or not by *Clostridium* species, which later develop under the cover of the Azotobacter slime. In the absence of both groups, the aerobic and the anaerobic, there is practically no fixation of nitrogen, even if some "oligonitrophilous" colonies are present. Generally, the writer did not succeed in finding in soil other nitrogen-fixing organisms except the above named. This suggests that the number of species, relatively numerous, described as fixing nitrogen are *not natural fixing agents*, although able to develop some power of fixation under the influence of artificial laboratory conditions.

The method of "large plates" presents decided advantages and it proved to give results more accurate and constant than those of fixation experiments under other conditions.

(a) The nitrogen-fixing flora of the soil sample develops all over the surface of the plate in the form of colonies or of centers of vegetation, which are easily determined both in quality and quantity. The direct count of the colonies gives the density of the cells, namely their number per gram of soil. This dose, if sampled *lege artis*, appears as truly representative of the respective non-symbiotic nitrogen-fixing flora.

(b) The activity of "the biological nitrogen-fixing apparatus" coming

directly from soil, unmodified, therefore, by cultural manipulations, shows, within experimental limits, a value so constant, as was previously never attained by using pure cultures.

(c) The constant yield of fixed nitrogen under these conditions renders possible establishing a *normal or standard process*, without which the determination of the activity of different soils can never be managed on a reliable basis.

(d) This activity must and can be characterized not only by the absolute gain or by the yield of nitrogen per unit of energy source, but chiefly by the *energy of the process*, namely, the time necessary for a determined gain; this period of time certainly depends on the density of the cells and their activeness. By a number of concordant experiences with samples of different origin, it was established that an active soil, in fixing 20 mg. of nitrogen, decomposes 2 g. of mannitol in 5 days (120 hr.); this makes one part of nitrogen fixed for 100 parts of mannitol (or glucose), or 1 part of nitrogen per 40 of organic carbon.

To determine the so-called "power of fixation" of soils, standard mannitol solution was universally used during a period of 20 years. The method is certainly untrustworthy in negative cases, and it is quite incapable of yielding the slightest information concerning the density of *Azotobacter* cells and their state of activity in the natural soil. The method may retain its historical value, but it is about time to replace it with more perfect methods, as the spontaneous culture and the silica plate methods, which are to be used *simultaneously*; the former giving indications chiefly concerning the activity and the latter concerning the density of the population.

These two characters are not necessarily parallel, as it might seem. Soil samples are frequently found which show on elective silica plates the presence of relatively numerous cells, but they refuse to give spontaneous cultures after addition of some mannitol. This fact is important, since it shows that *Azotobacter* germs, viable and easily developing on suitable medium *out of the soil*, keep obstinately at rest *in the midst of this soil*. What other interpretation of the fact can be suggested than that the soil has become infertile towards the nitrogen-fixing flora that it harbors? It is evident, therefore, that a soil containing an inactive specific flora cannot be considered as active. On the basis developed above, four categories of soils may be tentatively established.

#### TENTATIVE SOIL CATEGORIES

(1) *Soils very active*: These give in 48 hours at 30° rich spontaneous cultures, both microscopic and macroscopic; on the large plates they show the maximum number of centers of vegetation of *Azotobacter* (2500–3000); they attain easily the standard gain of nitrogen in 120 hours.

(2) *Soils not very active*: These give slower spontaneous cultures which

are considerably less abundant; they form on large plates about a hundred to a thousand centers; they show a gain slightly but constantly lower than the first group of soils.

(3) *Soils temporarily inactive*: They give no spontaneous cultures of any kind; they form on plates a few to some hundred centers; the gain in nitrogen is inferior to standard of 10 per cent or more.

(4) *Soils permanently inactive*: These give no spontaneous cultures of any kind and no *Azotobacter* colonies on plates; they give no gain of nitrogen or mere traces, not exceeding some decimilligrams.

The writer believes that it may prove useful in practice to submit soils to periodical tests by means of these rapid and easy methods, to get information concerning their state of activity. Agriculturists avoiding microscopic work might use the starched soil plates, which give nearly parallel results with the microscopic method.

But still further conclusions can be drawn from these methods. The existence of soils not very active and temporarily inactive,—that is, soils harboring cells but giving them no fair chance of development,—places the question concerning the means of restoring to them the lost activity.

The addition of soluble phosphates and of carbonate of lime was tried and decisive results were obtained. A set of experiments soon to be published has shown especially, that in soils where the scarcity of available phosphate is the limiting factor, there is no easier and sharper method to indicate it than that of spontaneous cultures of *Azotobacter*.

The writer hopes that the general conclusion of this extensive trial of the direct method is that it is called to wider application, as promising a nearer approach to biological soil problems and a more effectual contribution therefore to Soil Science than the actual standard methods.

Whether it be equally convenient to apply it to groups less characteristic than the above treated, remains to be seen. In all cases, however, where this application will not meet with serious difficulties, the method can be expected to lead not only to hypothetical or conventional, but to more real knowledge.

#### LITERATURE CITED

- (1) Winogradsky, S. 1925. *Ann. de l'Institut Pasteur*, 39: 299.
- (2) ———. 1926. *Ibid.* 101: 455.

# THE DIRECT MICROSCOPICAL AND BACTERIOLOGICAL EXAMINATION OF AGRICULTURAL SOIL

G. ROSSI AND S. RICCARDO

*Royal Superior Agricultural Institute, Portici, Italy*

Certainly it is not exaggerating to assert, as we have already done several times, that a bacteriological doctrine of the agricultural soil does not as yet exist, in as much as what we know about the quantity and quality of the microbial flora of the soil leads to the discouraging conclusion that, if we know some microscopic species that reproduce *in vitro* certain processes known to take place in the soil, we still cannot conclude that these forms are responsible for the transformation in the soil. These few individuals, and often so few, that are isolated may often manifest their activity only after a period of enrichment.

The principal reason for this condition is the fact, that in the study of the agricultural soil the ideas have erroneously been an outgrowth of the ideas acquired from the subject of the bacteriology of water instead of developing in terms of the true soil conditions which, in their heterogeneity approach animal and vegetable tissues. Therefore, we believe that much is to be expected from the direct examination of the soil.

Direct examination was initiated by J. Conn who discovered a method of staining the microorganisms of the soil. The use of this method, however, has degenerated into mere counting of the soil organisms and even this application is far from receiving general acceptance. Winogradsky has made better use of the method but he has also limited himself to the study of stained suspensions of soils or products of centrifugation of soils. From such preparations he observed that all these manipulations did not destroy the colonies of microorganisms formed in the soil. He has given figures, more or less schematic of such residues.

We have tried to extend these direct observations and are glad to have obtained some results. The technical method used by us may be considered as being rudimentary at present as compared to the way it may later be modified. It consists essentially of a small machine with a support for a glass object which may be pressed against the soil and hold some of the soil particles and a number of bacteria. This is similar to what is called in bacteriology a preparation by contact with a colony (Klatsch-Praeparat of the Germans). The coloration is made with erythrosine or eosine in dilute alcohol containing phenol. Observations may be made with an exploring objective and then reexamined and magnified with an immersion objective.



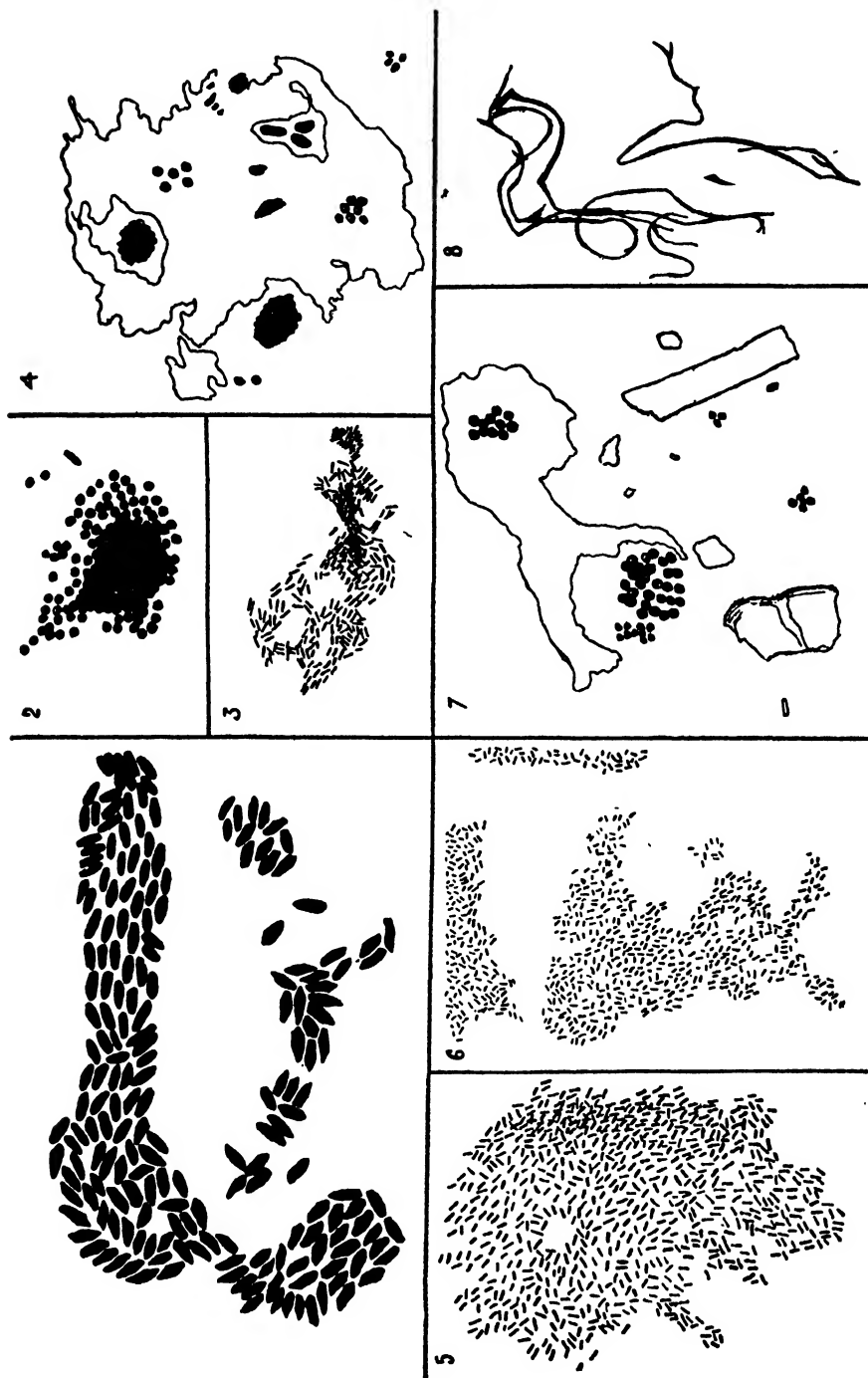


FIGURE 1

FIGURE 1.—The direct microscopical and bacteriological examination of the agricultural soil

*Magnification 1.* Ocular 4. Objective, 1/15 (Koristka).

*Magnification 2.* Ocular 3. Objective, 1/12 (Zeiss).

*Magnification 3.* Ocular 3. Objective, 5 (Koristka).

Soils: A. Mould of the Sussone Park (covered with dead leaves) of the Royal Superior Agricultural Institute of Portici (Italy).

B. Mixed soil of the Sussone Park (not sifted).

C. Sifted soil in pot (mixture of 4/5 of mould mentioned above and of 1/5 of Vesuvian subsoil).

D. Clayish soil of Montecorvino Rovella (Salerno). From preparations by impression from soil *in situ* dyed with phenolated erythrosine. The black corresponds to the red of the erythrosine, the zones surrounded in white had been dyed in light havana (colloids).

No. 1. Soil A. Magnification 2.

Nos. 2, 3, 5. Soil B. Magnification 2.

Nos. 4, 7. Soil D. Magnification 1.

No. 8. Soil C. Magnification 3.

Various experimental modifications permit one to take the impressions with a certain precision. Naturally a very large number of experimental treatments, more or less artificial, are applied to soils to prepare them for these impressions. From such preparations we have been able to examine the soil's bacteriological content actually and not metaphorically. This has had upon us the effect of a new world.

Colonies of cocci, bacteria, streptathrices and protozoa are more or less frequent in the agricultural soil. These colonies are morphologically definite, but totally different from those that we are in the habit of recognizing in artificial soils. They are mostly small (a few *microns*) and often mixed and filmy and provided with folds. They frequently take the form of rags, as if they developed within the irregular openings between the small mineral particles. The components of these colonies assume very often a morphology different from any that we have noted *in vitro*. Some accumulations appearing like colonies when seen at low magnifications are not clearly disclosed at high magnifications. This suggested the occurrence of ultramicroscopic forms. With a magnification of about 1000 diameters, filmy colonies are still obtained which are as large as the field of the microscope and perhaps even larger. A colony of *Streptothrices* (?) occupied as much as 14 fields at high magnification. The colonies of protozoa, more rarely than those of the *Schizomyceles*, are often in close association with the colonies of the *Schizomyceles*.

We may still say little of the distribution of the bacteria (or better of their colonies) in the soil and most of all of their presence and quality at a greater or smaller depth. We are under the impression that they diminish with the depth, but that they follow the roots of the plants. As regards the weather, we are inclined to believe that they vary considerably with the season.

As to the question of the isolated cells of the bacteria it seems to us that they are in the course of forming colonies or are arising from colonies. It is probable that the greater part of the colonies become disinterested by our manipulations, by reason of the present technic. These colonies which remain are still sufficiently numerous to give some information. Naturally the soils in which more colonies were found were those containing more organic matter undergoing decomposition as, for instance, the mould of woods or the earth obtained from mixing artificially the latter with poor earth. In pure clay soils colonies are also found. It has not been possible, however, except in a few cases as that of *Azotobacter* to identify the colonies. It seems to us that all of the bacteria that become stained, whether single cells or not, being alive, after their death in the soil they decompose and disappear just as they do in our cultures and as do all vegetables in the soil.

Naturally each of these simple assertions is only an index of a problem open to us and to other experimenters. However, it is believed that these

observations (and the whole development to be expected from them) will be the best material for answering the fundamental question of the existence or absence of an association of soil microorganisms.

We also believe that artificial conditions have too often been confused with those which occur in the natural soil (understanding by soil that which remains immobile for years, for centuries, for several thousands of years, in the woods, in the tundra, in the steppes, in the savannes (prairies) with what is forming from year to year). In these soils the climate exerts influences according to the season and the length of the day. The structure changes neither chemically nor physically and their relations with the higher plants are always static. Under these conditions the relations between different microorganisms reach more or less of an equilibrium as regards the influence of individuals upon one another as affected by their enzymatic activities, their products of decomposition and competition for food, oxygen and water. On the other hand, in the agricultural soils, this never happens if the agriculture is intensive, rarely and only partially in the pastures and in the fallow grounds, because here, harrowing, hoeing and various cultural methods modify at every instant the physical structure of the soil; chemical and green manuring at every instant modify the chemical structure; the sowing and the harvesting at every instant affect the biological conditions.

We are of the opinion that what we have observed should be considered as giving to the agricultural bacteriologists an indication that perhaps here has been found a new method for studying agricultural soil. Instead of observing agricultural soil from without we may begin to peer into the soil with the microscope. We hope that this direct study of the microorganisms of the soil, with a direction that we would call pedological may indicate to us the real importance and the true manner of the action of the microorganisms in the economy of the soil and in the whole circulation of matter. This may result in an immense profit to agriculture. It may further aid in the application of the biological laws, which will result in a complete transformation of these soil studies from an art into a science.

# THE NUMBER OF AMMONIA-OXIDIZING ORGANISMS IN SOILS

J. K. WILSON

*Cornell University, U. S. A.*

## INTRODUCTION

Numerous attempts have been made to determine the total number of bacteria in a definite portion of a soil, but as information has accumulated concerning the different physiological groups of soil organisms and their requirements such attempts have been largely abandoned. Yet it seems that definite information concerning certain organisms in the soil and the conditions under which they may be found might be of considerable value. The part which the ammonia oxidizing organisms play in the nitrogen cycle makes them of especial interest. Many articles may be found in the literature concerning the ammonifying and other groups but scarcely any dealing with the group that oxidize ammonia to nitrites. Manns and Gohcen (4) report the number of nitrifiers in one muck soil and in one peat soil in New Jersey. The soil with a lower calcium requirement, 4200 to 5200 lb. of calcium oxide per 2,000,000 lb. of peat, had a range of 10,000 to 33,000 nitrifiers per gram. The soil with a higher lime requirement, 12,600 to 17,000 lb. calcium oxide per 2,000,000 lb. of peat, had no nitrifiers when 1 g. quantities were examined. Razumov (5) reports the nitrifying bacteria in Russian soils to vary from 40,000 to 100,000 per gram.

Gainey and others have shown that the number of nitrogen fixing organisms (*Azotobacter*) varies with the reaction of the soil and Wilson suggested that the number of legume bacteria of certain physiological groups may dwindle in acid soils to a point where they may no longer supply the requirements of the plantlets. It would be valuable to know how the number of ammonia oxidizing organisms varies in soils in connection with the conditions under which they may be found. It was this idea that stimulated the investigation herein reported.

## EXPERIMENTAL METHODS

In making dilutions of soil suspensions it is possible to arrive at a point where a definite part of a gram may no longer contain an ammonia-oxidizing organism. Therefore, if a series of dilutions of a known soil are added to a sterile medium that is suitable for the growth of such organisms the by-product of them should accumulate and it should be possible to tell whether the organism is present or absent. This procedure has been adopted and the exact methods are outlined herewith.

## SECURING AND HANDLING OF SAMPLES

Samples were collected from soils on the University farm and these represented a variety of conditions. Samples were obtained of the upper 4 inches and were made up of 20 borings that constituted a composite. One exception to this was in the case of samples from Plat 3608. In this case soil was taken from 5 places and each treated as a composite. Any other exceptions to this are noted in connection with such experiments. From the composite 100 g. were used for moisture determinations. The rest of the sample served as material from which dilutions were made. Enough of the sample was taken to obtain 100 g. of dry soil. This was triturated with water, made to definite volume, shaken 400 times, and dilutions made from the muddy suspension. All dilutions were made with sterilized water with the aid of sterile pipettes. Care was exercised throughout the work to avoid contamination from any source. The final dilution, representing a definite amount of soil was the inoculum for a sterile medium within a container. The dilution from each soil representing the greatest quantity of soil used was tested for the absence of nitrates and nitrites to be certain that later tests would be accurate.

Suspecting that soils may be deficient in ammonia and that this and other factors would control the number of organisms in the soil, it was difficult to estimate what quantities of soil should be taken in order to establish a dilution where the ammonia oxidizing organism was present and where it was absent. As a result various quantities of soil suspensions were used. The largest amount was equivalent to 1 g. of soil and the smallest was equivalent to one five-millionth of a g. As information was obtained concerning the numerical presence of these organisms in a soil it was possible to use a smaller number of dilutions and to narrow the range. These various portions were used in triplicate.

## CHOICE OF MEDIUM

When Winogradsky isolated the organism that produces nitrites from ammonia he used both a liquid and a solid medium, the only difference in the two being that silica gel was added for solidification purposes. Each had as its basis inorganic constituents. Manns and Goheen (4) used in their determinations of the number of nitrite producing organisms, a solid medium containing not only inorganic substances but also 1 per cent mannite. They report the nitrite producing organisms as brown colonies around which were abundant nitrites as determined by proper reagents. Von Sack (6) used both solid and liquid media in isolating nitrite organisms that were apparently not identical with those discovered by Winogradsky. Joshi (2) also used modifications of the medium suggested by Winogradsky to isolate what he called a new nitrite producing organism. It may be, therefore, that no one medium will be suited to all the ammonia-

oxidizing organisms in soil and that no one medium will bring out the total number of these organisms that may be present.

In order to select a medium that would give uniform results four media were tried. Varying quantities of a soil were added to them. After a period of time a test was made for nitrites. From the information obtained one medium was selected and used throughout the remainder of the work.

#### MEDIUM USED

The medium finally selected for the work was made as follows. About 50 g. portions of ground limestone that gave no test for nitrates or nitrites were put into 200 cc. Erlenmeyer flasks. The flasks were plugged with cotton and sterilized. To the limestone in each flask was added about 12.5 cc. of the following sterile solution:

	grams
Ammonium sulfate	1.0
Dibasic potassium phosphate	1.0
Magnesium sulfate	0.5
Sodium chloride	2.0
Ferrous sulfate	0.4
Distilled water	1000 cc.

#### INCUBATION OF CULTURES

After each flask had received its quantity of the soil suspension it was thoroughly shaken and then put into an incubator. The incubator was kept at room temperature which had a range of temperature from 20 to 25° C. At the end of the seventh and fourteenth day the cultures were again shaken. On the twenty-first day they were tested for nitrites. Each section of the incubator held 36 flasks and as it was kept tightly closed there was but little loss of water from the flasks. Thus it was unnecessary to replace water at any time.

#### TESTING FOR NITRITES

At the end of the incubation period the cultures were examined for nitrites which were to be the criteria of the presence or absence of the ammonia-oxidizing organisms. Each culture was extracted with 25 cc. of nitrite-free water and the extract filtered through nitrite-free filter paper. To this filtered extract was added sulphanilic acid-alpha-naphthalamine, the reagents for detecting nitrites. The starch iodine test was also tried but did not seem to be suited to this work.

#### DETERMINING SOIL REACTION

The soil was extracted with about  $2\frac{1}{2}$  times its own weight of boiled water and the extract filtered through washed filter paper. The pH value of the clear or opalescent solution thus obtained was determined colorimetrically.

## THE DETERMINATION OF AMMONIA-OXIDIZING ORGANISMS

After several determinations of the number of ammonia-oxidizing organisms in samples from various field plats had been made and confidence established in the methods of work, other samples were collected and dilutions made as previously outlined. These were collected on December 2. The samples were taken to the laboratory, well mixed, and moisture determinations made. After a representative portion was weighed for moisture the remaining sample was placed in the ice box until the following day. On December 3 the dilutions were made and incubation started. On December 24, tests were made to determine the presence or absence of nitrites which were the criteria of the presence or absence of the organisms. The results are given in Table 1.

*TABLE 1.—Part of gram in which ammonia-oxidizing organisms were present  
Samples collected December 2, 1926*

Plat No.	Reaction of water extract	Moisture in sample	Part of gram in which organism was	
			Present	Absent
	pH	per cent		
Ab	8.2	30	1/1,000,000	1/5,000,000
B	5.2	20	1/5000	1/6200
729	5.4	20	<sup>a</sup>	1/1500
752	7.0	20	1/5000	1/6200
755	5.4	20	1/8260	1/10,000
2114	7.0	24	1/40,000	1/60,000
3608—I	6.2	20	<sup>a</sup>	1/1000
II	6.4	20	1/3500	1/4170
III	6.6	20	1/6280	1/8260
IV	6.8	20	1/25,000	1/50,000
V	7.0	20	1/35,000	1/50,000
3610 <sup>c</sup>	7.0	23	1/5000	1/6250
3611 <sup>c</sup>	7.0	23	1/10,000	1/20,000

<sup>a</sup> Not large enough quantity used.

<sup>b</sup> Place on which manure was annually composted.

<sup>c</sup> Seeded in 1922 to orchard grass and to alfalfa respectively.

It is clearly evident that the numbers per gram of soil of ammonia-oxidizing organisms vary greatly. They vary from less than 1000 in the soil of Plat 3608—I to more than 1,000,000 in soil on which residues and manure had been composted. It is also noted that the number per gram decreases as the soil becomes more acid. The number in soils from Plats B, 729, 755 and 3608—I whose pH is 5.2, 5.4, 5.4 and 6.2 respectively was about 5000, 1500 or less, 8260 and 1000 or less per gram



respectively. Also the soil from a place where residues and manure has been annually composted contained at least 1,000,000 such organisms per gram. This soil had a pH of 8.2.

The samples from Plat 3608 were taken 3 feet apart, sample I and V having been taken only 12 feet from each other. This plat was seeded to alfalfa in 1922. When samples were collected alfalfa had very largely disappeared from the most acid end of the plat, leaving a rather sharp line where alfalfa would grow and where it would not grow. On this line the soil was pH 6.6. It is noted that sample I with pH 6.2 had less than 1000 per gram, sample II with pH 6.4, 3500 per gram, sample III with pH 6.6, 6280, sample IV with pH 6.8, 25,000 and sample V with pH 7.0, 35,000 per gram of soil. This is a perfect correlation of pH and the presence of ammonia-oxidizing organisms.

Plat 3610 was seeded to orchard grass and 3611 which lay alongside it to orchard grass and alfalfa. This was done in 1922. The number of ammonia-oxidizing organisms per gram of soil from these two plats determined in December, 1926 was 5000 and 10,000 respectively. The reaction of the soil from the two plats was the same. This suggested that there may be an effect of the crop on the number of such organisms.

## INFLUENCE OF CROPS ON THE NUMBER OF AMMONIA-OXIDIZING ORGANISMS

In order to test the influence of crop plants on the number of ammonia-oxidizing organisms, quadruplicate samples were taken on January 13, 1927 from Plats 3610 and 3611. At this time about 8 in. of snow covered the ground. The soil was not frozen enough to interfere in collecting unfrozen soil. Sample 1 from each plat was taken about 5 feet from the adjoining edges of the plats, thus these two samples were only 10 feet apart. This is also true of samples 2, 3 and 4 for the two plats respectively. Table 2 shows the results.

TABLE 2.—*Number of ammonia-oxidizing bacteria in soil growing orchard grass only and orchard-grass and alfalfa*

	Number of ammonia oxidizers per gram sample			
	1	2	3	4
Orchard grass only	6000	4000	8000	12,000
Orchard grass and alfalfa	6000	8000	9000	13,000

These data show such slight differences in the two plats that it is doubtful whether one should conclude that different kinds of crops, at least in this case, influence the number of ammonia oxidizers in the soil. The re-

action of all samples was pH 6.8 to 7.0. The moisture was 21 per cent in the orchard grass and alfalfa plat samples and about 25 per cent in those from the orchard grass plat except sample 1 which had about 35 per cent moisture.

Other determinations were made of the number of ammonia-oxidizing organisms in soils that had been variously cropped. Samples were taken on August 2, 1927 from a series of plats. The data are given in Table 3.

TABLE 3.—*Influence of previous crop on the number of ammonia-oxidizing organisms when soils are growing different crops*

*Samples August 2, 1927*

Plat	Moisture in sample	Previous crop	Present crop	Reaction	Part of gram in which organism was present or absent	
					Present	Absent
	per cent			pH <sup>a</sup>		
2002	18.5	Alfalfa	Timothy	7.45	500,000	<sup>b</sup>
2003	15.0	Do	Corn	7.45	500,000	<sup>b</sup>
2007	19.0	Do	Alfalfa	7.45	500,000	<sup>b</sup>
2008	15.0	Do	Corn	7.69	500,000	<sup>b</sup>
2012	18.5	Timothy	Alfalfa	7.73	500,000	<sup>b</sup>
2013	16.5	Do	Corn	7.46	100,000	250,000
3003	17.5	Rye and Vetch	Oats	7.53	100,000	250,000
3004	17.5	Vetch	Do	5.81	75,000	100,000
3005	20.0	Rye	Do	5.41	75,000	100,000
3006	17.5	Rye and Peas	Do	5.74	35,000	50,000
3007	18.5	Peas	Do	5.74	100,000	250,000
3008	20.0	Rye	Do	6.16	100,000	250,000
3009	17.5	Oats and Vetch	Do	6.80	75,000	100,000
3010	18.5	Oats	Do	6.65	35,000	50,000
3011	18.0	Rye	Do	5.83	35,000	50,000
3012	18.5	Oats and Peas	Do	5.66	50,000	75,000
3013	18.0	Buckwheat	Do	5.66	35,000	50,000
3014	20.0	Rye	Do	5.46	75,000	100,000

<sup>a</sup> Determinations made by the quinhydrone method.

<sup>b</sup> Not small enough quantity taken.

It will be noted that the moisture content of these samples did not differ very greatly. Also there is no striking correlation between the previous crop and the presence of ammonia-oxidizing organisms. But if one looks at the reaction of the soil and compares it with the number of ammonia-oxidizing organisms there seems to be a correlation. In two cases out of seven, where the soil has a reaction greater than pH 7.0 the number of such organisms is below 500,000 per gram, while there are only two cases out of eleven where the soil has a reaction lower than pH. 7.0

that the number of such organisms is as high as 100,000 per gram. Many of the 11 samples have a very much smaller number than 100,000 per gram. It would seem from these data that if the previous crop has an influence on the number of ammonia-oxidizing organisms these samples were taken too late to show such an influence.

Two desiccated soils were also tested for the presence of ammonia-oxidizing bacteria. These were soil samples that had been air-dried in 1925. They were obtained from Tanks 11 and 12 of the University lysimeters. Both soils were for a number of years in a rotation of corn, oats, wheat, timothy and timothy, also both were fertilized at the rate of 200 lb. per acre with potassium sulfate and farm manure at intervals at the rate of 7 tons annually. The only difference in the two samples is that soil from Tank 12 had received burned lime so that its reaction was about pH 7.2. The reaction of the soil from Tank 11 was pH 6.6 to 6.8. The number of ammonia-oxidizing bacteria in the desiccated soil from Tank 11 was not much over 1000 per gram while those in the soil from Tank 12 was about 5000.

These results are from only the two samples and may not represent true conditions, however, they clearly indicate that desiccated soils still contain a sufficient seeding of these organisms to start active ammonia-oxidation once they are brought under favorable conditions.

## DISCUSSION

The results of this study of the number of ammonia oxidizing-organisms in field soil leads to the conclusion that there is a wide variation in the number of such bacteria in the soil, and that they are reduced in numbers as soils become more acid. In a soil that has a reaction of pH 6.2 there may be only one-thirtieth to one-fiftieth as many ammonia-oxidizing organisms as the same soil may have if it has a reaction pH 7.0.

These results are similar to those obtained by the author (7) in a study of the number of legume bacteria in these same soils. For convenience of comparison the following summary of a part of these data is presented.

TABLE 3.—Variation in bacterial numbers per gram of soil of ammonia-oxidizers and of *Bacillus radiculicola* of certain legumes with change in soil reaction. Plat No. 3608

Sample No.	pH	Ammonia-oxidizers	<i>Bacillus radiculicola</i>		
			For medicago	For trifolium	For vicia
I	6.2	Less than 1000	None in 5 g.	100,000	1000
II	6.4	3500	Do 5 g.	100,000	1000
III	6.6	6280	10 per g.	100,000	1000
IV	6.8	25,000	1000 per g.	100,000	10,000
V	7.0	35,000	1000 per g.	1,000,000	10,000

This summary indicates that both the ammonia-oxidizers and the legume bacteria increase in numbers as the acidity of the soil decreases. This might indicate that either the acidity as such is toxic to these organisms or that the more acid the soil the less available is the organic and inorganic substances in the soil. The fact that organic residues decompose more slowly in very acid soils than in a neutral or slightly acid one suggests that in acid soils there is a competition for basic nitrogenous substances in which the soil plays a prominent rôle. The absorption of bases by acid soils might indicate that they are deficient in bases and that as fast as the organic materials become mineralized the bases, including basic nitrogen compounds, enter into combination with the colloidal nucleus probably replacing hydrogen and become somewhat unavailable to organisms. Ashby (1) is of this opinion and says that both clay and peat absorb the ammonia of ammonium salts, the action being a substitution whereby the ammonium ion displaces another base probably hydrogen from its combination with hydrated aluminum silicate or humic acid and that such a combination yields no ammonia to pure water. These absorbed nitrogen compounds are probably not nitrifiable until they are liberated from the soil complex and changed to ammonium carbonate, this latter alone being oxidizable by the autotrophic bacteria.

If in the decomposition of organic matter which precedes ammonia oxidation such compounds as amines, alkaloids, purine bases, etc., are presented to an acid soil they may function not only as bases but also may be toxic to the ammonia-oxidizing bacteria as well as to the soil flora in general. This toxic condition may also be reflected in a decreased number of those organisms that oxidize ammonia to nitrites.

Also if ammonium-carbonate alone is nitrifiable and this process is delayed, as is usually supposed, until the readily available organic material has been oxidized to carbon dioxide and water it would seem that there may be a deficiency of carbon dioxide in the soil solution in very acid soils, especially if the soil is bare or recently planted. This deficiency may retard the formation of ammonium carbonate and may also be reflected in decreased numbers of those organisms that oxidize ammonia. In addition carbon dioxide is essential in the process for it serves as a source of carbon for these autotrophic organisms.

One might expect to find more ammonia-oxidizing bacteria where plants are growing than otherwise and that they would be more numerous under crops like legumes than under certain others. If the accumulation of nitrates in soils, which apparently is preceded by the formation of nitrites, can be taken as an indication of the numerical presence of these organisms then the work of Lyon and Bizzell (3) strongly suggests that more ammonia oxidizers may be found under such crops as legumes and corn than under timothy and oats. The determinations of the number of such bacteria in a soil well established to orchard grass and alfalfa that are reported in this paper are in accord with this suggestion.

The very great increase in carbon dioxide in soils that are planted to crops may indicate a greater mineralization of inorganic constituents of the organic matter. Such constituents being returned to the soil solution that is heavily charged with carbon dioxide, in all probability, other conditions being equal, should be absorbed by the soil in order of their electromotive displacing force. Ammonia, which is very low in this respect, should not be taken out of the soil solution as quickly or as permanently as potassium, calcium, magnesium, etc. This may result in more ammonium carbonate in the soil solution which can be oxidized to nitrites and this would be reflected in a greater number of the organisms in question.

### SUMMARY

Soil samples were collected from field plats and examined for those bacteria that oxidize ammonia to nitrites. Definite portions of these soils were used as an inoculum for a sterilized medium that had been tested for its suitability to the bacteria in question. After proper incubation at 20 to 20° C. for 21 days the medium was tested for nitrites. These were the criteria for the presence or absence of the organism in the soil.

Soil samples thus handled showed a wide variation in the number of ammonia oxidizers. They varied from only a few hundred to more than a million per gram of soil. This variation went hand in hand with the reaction of the soil. Soils whose reaction was around pH 5.4 supported fewer such organisms than those whose reaction was pH 7.0. No soils were studied whose reaction was more alkaline than pH 8.2.

Desiccated soil samples contained viable ammonia-oxidizing bacteria, more being found in a neutral or slightly alkaline soil than in an acid soil.

These findings are in accord with studies previously reported of the number of legume bacteria in these same soils.

### LITERATURE CITED

- (1) Ashby, S. F. 1907-08. Some observations on "nitrification." *Jour. Agr. Sci. [U. S.]* 2: 52.
- (2) Joshi, N. V. A new nitrite-forming organism. *India Dept. Agr. Mem., Bact. Ser.* 1: 85.
- (3) Lyon, T. L., and Bizzell; J. A. 1913. Some relations of certain higher plants to the formation of nitrates in soil. *Cornell Univ. Agr. Expt. Sta. Mem.* No. 1.
- (4) Manns, T. F., and Goheen, J. M. 1916. A preliminary report on muck humus as a fertilizer and carrier of beneficial soil bacteria. *Delaware Agr. Expt. Sta. Bul.* 115.
- (5) Razumov, A. S. 1926. Method of counting the bacteria in soil according to their physiological groups. *Expt. Sta. Record*, 55: 319.
- (6) V. Sack, J. 1925. Eine nitrithildende Bakterie. *Centrabl. Bakt. [etc.]. Abt. II*, 64: 32.
- (7) Wilson, J. K. 1926. Legume bacteria population of the soil. *Jour. Amer. Soc. Agron.* 18: 911.

# THE ADVISABILITY OF STANDARDIZING THE METHODS USED FOR THE QUANTITATIVE DETERMINATION OF SOIL BACTERIA AND THE CHANGES PRODUCED BY THEM

A. G. LOCHHEAD

*Dominion Experimental Farms, Ottawa, Canada*

## INTRODUCTION

There are probably very few students of soil microbiology who have not, at one time or another, felt certain misgivings as to the value of continuing their work in their chosen field; who have not at times been beset with grave doubts as to whether soil microbiological studies are, to put it briefly, worth while. Many workers, after the initial enthusiasm of work in the new field has worn off, discover, to their chagrin, that the revolutionizing of agriculture in their generation is impossible. Coming gradually to a realization that the practical application of the results of investigation in soil microbiology is inevitably an exceedingly slow process, many have felt half inclined to abandon soil work, and turn to other fields where microbiology rests on a more secure footing, and where research and application do not appear to be quite so severely estranged.

Some of our soil bacteriologists have indeed left the field to employ their time and energies in some line of microbiological research where investigations appear to lead to results of a more tangible nature, being impelled by their own personal outlook as to the function of research, or compelled by more materialistic considerations in cases where employers insist, all too ignorantly, on what are called "practical results."

That of late years a certain degree of pessimism as to the utility of soil microbiological research has been widespread is no doubt apparent to most of us. This fact has been brought out by Dr. Waksman (6) in the report of his survey made in 1924, in which he has tried to feel the pulse of soil microbiology in many different regions. The science of soil microbiology is popularly believed and frequently stated to be still in its infancy. Many of us have probably wondered at times whether it is to be like Peter Pan, and never grow up! In the report just referred to, Dr. Waksman even poses the question as to whether such a science can be said to exist at all at the present time (1924). Dr. Waksman has, I think, helped to answer the question himself, and the recent appearance of his "Principles of Soil Microbiology" has given to the world a definite announcement that the infant has at least been born.

Yet in spite of the doubts which assail many of us at times, it cannot be denied that the soil offers a very alluring field for study, is drawing recruits in increasing numbers, and will continue to attract workers for whom the simple desire to understand more fully the complex phenomena of the soil is sufficient reason for engaging in research.

The apparent paucity of practical results in the domain of soil microbiology is due in the largest measure to the infinite complexity of the soil itself. The worker in this field is confronted at the outset with much greater difficulties, in part, than his colleagues working in dairy, industrial and medical bacteriology. Unlike the latter, he is unable to work, apart from tests *in vitro*, with pure cultures, and if he attempts to do so with his subject proper (by resorting to the present known methods of sterilization) he is dealing with something quite unlike his original soil. When we consider that, in addition to the handicap arising from the difficulties inherent in soil itself, soil microbiology has been able to attract but few workers in comparison to medical bacteriology, it is not to be wondered that, as a science, it has not been able to pass quickly out of its infancy.

### THE NEED FOR UNIFORMITY OF METHODS

There will be, I think, general agreement as to the necessity of making the work now being done in soil bacteriology count for the most. If any science is universal soil science undoubtedly is, and consequently the future structure of soil microbiology will be of necessity built up through the united efforts of workers in all parts of the globe. At the present time it is unfortunately true that in no branch of science is there to be found less general uniformity of method than in soil microbiology. This lack of uniformity is particularly apparent in the case of quantitative estimations of soil bacteria and other microorganisms, and in the measurement of the changes brought about by the action of these various microorganisms. With such a state of affairs it is reasonable to presume that we are not gaining as much as we might from the work being done at present, and that the science is not forging ahead as quickly as it might if the results obtained by widely scattered investigators were more readily comparable. How often have we wondered, in reading the report of a distant colleague's work, just how closely the results coincided with our own obtained in a similar type of investigation, and how delighted we are to find that by some chance, some other distant colleague's methods were similar to our own. Too often the value of some outstanding piece of research is lessened by the fact that through diversity of methods employed in various quantitative determinations, it cannot be correlated with work from other sources dealing with the same subject.

Variations in methods are by no means concerned with international boundaries. Even within the same countries differences in technique

employed in different laboratories in connection with microbiological and biochemical methods render data from these various sources but to a limited degree comparable. A simple illustration in support of this point may be drawn by a comparison of methods for making total plate counts of soil organisms, as described in papers appearing in the journal "Soil Science" for a few years back. In 16 articles, ordinary plate counts of soil bacteria were with 8 different culture media. Only one medium found favor with more than one author, in which exceptional case the same medium was used in 9 instances. This apparent preference, however, is accounted for largely by the fact that the majority of the papers in which it is described were issued from the same laboratory. The 16 papers really represented 10 different laboratories, so we really have 8 different media from 10 sources. The same general lack of uniformity is apparent with factors affecting the plate counts other than the media, such as temperature and time of incubation. The temperature employed ranged from room temperature to 37° C. while the incubation period varied from 4 to 17 days.

Other factors which enter into the technique of quantitative estimations of soil bacteria, such as the manner of sampling the soil, the preparation of the various dilutions, the numbers of parallel plates made, need not be here elaborated. The importance of taking such factors into consideration and the general lack of uniformity in this regard have been already stressed, notably in a paper by Northrup-Wyant (3). The author emphasizes the need for better uniformity and points out that "an effort towards a standardization of certain fundamental features of the technique would be a desirable step."

The lack of a definite set of standard methods is apparent, not only in the case we have cited, but in almost all our technique involving quantitative estimations of soil microorganisms, of particular physiological groups, and in connection with biochemical measurements of changes produced by soil organisms. Not only in the matter of the enumeration of organisms have we this diversity of method, but also with such procedures as those involving an estimation of the relative nitrifying power or the nitrogen fixing capacity of soil samples. Apart from the question of their absolute worth, it will perhaps be granted, even by those who see little or no value in the commonly employed methods of biochemical soil analysis, that whatever value they might possess from a comparative standpoint would be enhanced through universal uniformity of procedure.

In a large measure, every investigator in soil microbiology is a law unto himself, with little uniformity to be discerned outside of the same laboratory. As a natural consequence, progress is necessarily slower than would be the case with more standardized methods enabling widely separated workers to be of more mutual aid.

There is probably no point on which soil microbiologists the world over



will be more generally agreed than that the methods for quantitative biological and biochemical soil analysis are sadly imperfect. With the best of media for our plate counts we obtain but a fraction of the bacteria known to be present in a soil. On any substratum yet devised numerous groups of important soil bacteria will be unrepresented. Organisms of the nitrifying and nitrogen-assimilating groups, of the cellulose-decomposing group, obligate anaerobes, and others will largely fail to develop on the substrata ordinarily employed for quantitative estimations. We have, furthermore, no certainty that an increase in the total numbers of bacteria in a given sample of soil will be reflected in an increased plate count. Indeed, should the increase be confined to groups which do not appear on the media employed, it is conceivable that, in view of the intricate systems of symbiosis and antibiosis in the soil, the plate count might well vary inversely with the actual totality of organisms present. The same type of objection may be applied in the case of our biochemical methods for estimating quantitatively the activities of particular groups of soil microorganisms. Here we are confronted with the difficulty arising from a failure to duplicate soil conditions *in vitro*, and are consequently unable to translate our results in terms of actual soil processes in nature.

#### ADVANTAGES OF STANDARDIZED METHODS

Having the foregoing in mind, it might well be asked, "What is to be gained by standardizing methods if our methods are admittedly imperfect?" Many, no doubt, will hold that the need for discovering improved, more accurate methods is much more urgent than the need for standardization; in other words the time is not yet ripe. It may be answered, in replying to such objections, that the potential worth of any particular analytical method cannot be evaluated until it has been tested as a standardized procedure. The quantitative estimation of soil bacteria by the plate method, for instance, is regarded in many quarters as being next to worthless in helping us to arrive at any understanding of soil processes, or affording an index of soil fertility. We think it is just possible that the plate method may disclose some hidden value if all soil bacteriologists could agree to use the same procedure and thus bring their results down to some common denominator. At all events, standardization would help us to arrive more quickly at some definite conclusion as to whether the procedure possesses any virtue at all. The same applies to any other quantitative method for enumerating soil microorganisms or for biochemical soil determinations. In spite of the lack of unanimity as to the value to be placed on any of our present-day quantitative methods, yet quantitative methods are inevitable in soil science and counts of organisms and estimates of their activity will always be made, and only when some uniformity of procedure exists shall we obtain the full worth, whatever that may be, of such methods:

In order that a scheme of standardized methods may be useful it is not necessary that the methods themselves be perfect. The perfect medium for cultivating bacteria or other microorganisms of the soil will probably never be discovered. Every medium that can be devised will be to a great extent a selective medium, allowing some types to grow and inhibiting other forms, and of the best media employed at present, that is to say, of those which give consistently the highest counts, the most that can be said is that they are rather less selective than other media.

Turning for a moment to other sides of bacteriological research; to dairy, water or medical bacteriology, we note a much less chaotic state of affairs in the matter of commonly accepted procedure for quantitative work. In these fields standardization has made some definite progress, and although it is by no means universal, yet, through its partial adoption, much good has accrued through the simple fact that data from one source may be compared with data from other sources, even though the procedure in itself may be open to criticism. In America, for instance, methods for the bacteriological examination of milk have been standardized, largely through the efforts of the American Public Health Association (1). The quantitative estimation of bacteria, however, is made on a medium of beef peptone agar, a substrate which is far from ideal for cultivating bacteria occurring in milk, and by no means one capable of yielding maximum counts. In spite of this handicap, however, the very fact of standardization has been of enormous value to bacteriologists and others concerned with the dairying industry and with public health.

Much the same can be said in regard to standard methods of water analysis employed in the United States and Canada. Even though procedures in themselves may be imperfect, yet whatever value they do possess is enhanced by standardization.

In soil microbiology, the adoption of any set of standard procedures would not mean that investigators would be discouraged from seeking better methods. Under a system of standardization the worth or worthlessness of a method would be much more quickly apparent than under the present system where we all have our favorite formulae, many of which could be well discarded. Any method considered worthy of adoption by soil biologists would be intended as provisional, to be superseded by a superior method when such has been commonly approved.

In many instances standardization would be easier than might be supposed. Frequently quantitative methods from widely separated laboratories differ in some comparatively minor point such as temperature or length of incubation, but still sufficiently to spoil their comparative value. We have all no doubt our own methods and procedures to which we prefer to adhere. For his part, however, the writer would gladly alter his thermostat to change the incubation temperature a couple of degrees, and willingly count plates or make a nitrogen determination a few days

later than usual, if, by doing so, he could align his methods in accordance with some scheme accepted by other bacteriologists.

### STANDARDIZATION OF METHODS BY SOIL CONGRESS

The International Society of Soil Science is an organization founded upon the principle of cooperation, and dependent for its success upon a continuance of the spirit of cooperation on the part of members drawn from all quarters of the globe. It is not surprising that at previous sessions of the society, workers in soil microbiology, with a realization that progress will only come with concerted effort, have stressed the need for greater cooperation in the field of microbiological research. Particular reference may be made to the pleas of Bargagli-Petrucci (2) and Rossi (5) on the occasion of the Conference of Soil Science at Rome in 1924, the latter particularly stressing the advisability of considering the question of standardizing methods and substrata for cultural work. The same author (4) on another occasion states his belief that a new impulse will be given to the study of soil bacteriology from the moment that scientists agree to adopt among other things standardization of methods of research.

If it is generally admitted that international cooperation is desirable, it would seem fair to assert that cooperation would be more feasible were we agreed on some measure of standardization. Standardization for quantitative study would bring a better mutual understanding, and would inevitably react favorably in aiding the progress of qualitative soil studies. It is certain that much work of a qualitative nature will be done in the future, and that much progress may be expected from intensive work on this hitherto all too neglected side of soil microbiology. Here, too, cooperation is essential if we are ever to hope for anything approaching a complete picture of the micro-population of the soil. Any degree of standardization, then, will undoubtedly give an impetus to cooperative studies of a quantitative and qualitative nature in what might be termed "pure soil science," that is to say, studies made without being influenced by the knowledge that some immediate practical end must be served. Fundamental research is assuredly the greatest need of soil biology, and if soil biologists will concentrate on such problems, the practical application will look after itself. Our crying need to-day is the study of soil bacteria as bacteria, of soil fungi as fungi, of the soil's microflora and fauna as interdependent organisms rather than as immediate aids to soil fertility.

It is not the aim of this paper to suggest any specific methods to be considered for adoption as standardized procedures. Its purpose is rather to suggest to microbiologists the desirability of considering whether or not the time is ripe to introduce standardized methods. In our opinion a beginning might at least be made. With this in mind two suggestions are offered:

(a) That on the occasion of this Soil Congress certain methods for quantitative study be adopted as provisional standard methods where there is sufficient unanimity concerning the value of these methods.

(b) That should methods be considered still too uncertain to be adopted at once, soil microbiologists and biochemists in as many different parts of the world as possible should undertake to compare methods as may be conveniently arranged, covering the various procedures employed in the determination of soil microorganisms and the changes produced by them. Results could be collected in anticipation of the next Soil Congress, for possible adoption of those considered most suitable.

#### LITERATURE CITED

- (1) American Public Health Association. 1923. *Standard Methods of Milk Analysis*, 4th ed., New York.
- (2) Bargagli-Petrucci, G. 1926. *Necessità di coordinare ed organizzare gli studi di microbiologia del suolo*. *Actes, 4th Confer. Internatl. Ped.* Rome, 1924, 3: 267.
- (3) Northrup-Wyant, Z. 1921. A comparison of the technic recommended by various authors for quantitative bacteriological analysis of soil. *Soil Sci.* 11: 295.
- (4) Rossi, G. 1923. The bacteriology of agricultural soil and its difficulties and fallacies. *Internatl. Rev. Sci. and Pract. of Agr.* N. S. 1: 14.
- (5) ———. 1926. *Proposta per una intesa fra tutti i batteriologi agrari del mondo per lo studio sistematico dei terreni agrari locali*. *Actes 4th Confer. Internatl. Ped.* Rome, 1924, 3: 285.
- (6) Waksman, S. A. 1925. Soil microbiology in 1924: an attempt at an analysis and a synthesis. *Soil Sci.* 19: 201.

# THE PRESENT POSITION OF OUR KNOWLEDGE OF THE DISTRIBUTION AND FUNCTIONS OF ALGAE IN THE SOIL

B. M. BRISTOL-ROACH

*Rothamsted Experimental Station, England*

## INTRODUCTION

The study of soil algae has been approached from two very different points of view, which put an adequate summary of the systematic literature on the subject quite beyond the scope of a short paper. According to the first point of view the soil is regarded as merely a special case of a much wider class of habitats, including the surfaces of rocks, tree trunks, palings, the roofs and walls of buildings, etc., in which the dominant climatic condition against which the algae have to contend is an erratic and often inadequate water supply, coupled sometimes with the further deleterious effects of direct insolation. Algae growing in such habitats have attracted attention for many generations, and the literature dealing with these subaerial, or, as Fritsch prefers to call them, "terrestrial" algae, is quite extensive. Their significance in causing the erosion of rocks, building materials, etc., has been pointed out, as has also their importance in forming the first thin layer of humus on uncolonized surfaces which prepares a foothold for the later appearing fungi and lichens. Some of the most interesting recent work carried out from this point of view is that of Fritsch and his coworkers on the moisture relationships of certain terrestrial algae, in which it is shown that the protoplasts of the cells of these algae exhibit a paucity or even a complete absence of large vacuoles, most of the sap being dispersed through the cytoplasm, and much of it being retained by the cell in the air-dry condition. It is also shown that these algae require relatively little moisture to replace that lost by the protoplast in drying, and that appreciable amounts of this can be absorbed from the atmosphere at times when the humidity of the air is great. Further, such terrestrial algae respond to changes in humidity of the atmosphere more rapidly than does inanimate material, and at times of low humidity the rate of loss of water from the cells falls off much more quickly than in non-living material. They explain the characteristic behavior of these algae towards moisture largely on the basis of the high concentration of solutes in the cell sap, for they found that the cells show on the whole an abnormally high resistance to plasmolysis by hypertonic solutions, a condi-

tion which I am able to confirm from my own observations on certain species of algae isolated directly from soil.

Workers approaching the subject from this first point of view therefore emphasize the importance of water supply and assume at least an adequate illumination, drawing attention to the formation in exposed situations of screening pigments which serve to protect the protoplasm from the harmful effects of too great a light intensity. The second point of view, on the contrary, regards the true soil algae as regular inhabitants of the soil itself, capable of growth both on the surface and below ground, where the question of resistance to desiccation becomes of minor importance in comparison with the more urgent one of carbon nutrition in the absence of light. In fact, in view of the nature of the soil substratum with the continuous rise of water by capillarity from the lower layers, even during the driest periods, it is very unlikely that the soil algae are ever subjected to such extremes of drought as may often befall the subaerial algae; and ability to adopt a saprophytic habit of nutrition becomes the dominant factor in determining the survival of these organisms below the surface of the soil.

The systematic work that has been carried out on the true soil algae has revealed the fact that these organisms are universally present in cultivated soils both on the surface and to quite considerable depths, and that though some types of soils are richer than others in species yet certain definite species appear to be of very widespread distribution. *Protococcus viridis* Ag. and *Heterococcus viridis* Chod, two unicellular green forms have been obtained in Central Europe, England and America, while *Chlorococcum humicola* or allied species and *Hantzschia amphioxys* appear to be ubiquitous. In uncultivated soils, on the other hand, algae are fewer on the surface and except in very favorable conditions of moisture are absent from the lower layers or present in very small numbers.

Francé, working with soils from central Europe, has recorded diatoms as forming the chief constituents of the soil population in many localities, but this is not the experience of the writer nor of Chodat nor Moore and Carter who have recorded a preponderance of green algae, mainly unicellular forms, in the soil samples examined by them from England, Switzerland, China, Malay, Sudan and America. In warmer climates, especially those with a humid atmosphere, it is probable that the blue-green algae may play a dominant rôle, as is suggested by the fact that Esmarch for central Africa and Brühl and Biswas for India almost confine their attention to these forms. Unpublished data obtained from Rothamsted soils suggest that the relative frequency of blue-green forms in cultivated soils in England may be partly determined by the crop grown, and the varied cultural operations which are consequently applied to the soil; soil bearing a root crop requiring that the surface should be kept in a state of fine mulch was found to be very deficient in blue-green forms, though a similar

soil bearing a permanent wheat crop was rich both in species and in individuals, whereas the green algae and diatoms were almost identical in the two soils.

### DISTRIBUTION OF ALGAE IN THE SOIL

The quantitative examination of soil samples from different depths at Rothamsted has shown that the alga-flora is distributed laterally fairly uniformly within a limited area in a cultivated soil, and that the species found in the lower depths are mainly identical with those on the surface. In point of numbers of individuals there is considerable horizontal stratification within the soil; the numbers are usually high in the top inch, significantly lower in the second and third inches, high at the fourth inch depth where they are often equal to or possibly higher than on the surface, and considerably lower at the sixth inch; while at the twelfth inch depth the numbers are few and since the subsoil had already been reached it is probable that the majority of these were in the form of resting spores.

Some idea of the numbers of individuals present at different depths may be obtained from two series of samples taken vertically below one another from the dunged and unmanured plots respectively of Broadbalk wheat field in the summer of 1920. The first series taken on June 28th from the unmanured plot contained approximately 16,000 algae per gram of soil in the top inch, 10,000 in the second inch layer, 28,000 at the fourth inch depth and only about 4,000 at the sixth inch depth. The same plot a month later was found to contain nearly 22,000 algae per gram of soil, while a series of samples taken on the same day from the dunged plot showed a very much denser algal population, viz. approximately 62,000 per gram of soil in the top inch, 28,000 in the second inch, 56,000 in the fourth inch and nearly 15,000 at the sixth inch depth.

Under less favorable conditions for growth the numbers have been observed to fall very much below these figures, records as low as 700 per gram of soil having been obtained for a 1 to 6 inch sample during the summer drought of 1921, while after a fortnight's severe frost in November 1920 the numbers were reduced to 90 per gram in the top inch, 150 in the second inch, 500 at the fourth inch and only about 50 at the sixth inch depth.

The large numbers of individuals at the fourth inch depth correspond to the region of greatest biological activity as established for bacteria and other groups of soil organisms.

### METHOD FOR DISTINGUISHING BETWEEN RESTING AND VEGETATIVE CELLS

Until recently the condition in which the algae exist in the soil had been a matter for speculation, and though the physiological behavior of kindred algae had often been quoted in favor of the probability of their

existence in the vegetative condition yet direct evidence on the subject was not available. During the past summer, however, the writer has devised a method by which a distinction may be drawn between the resting and the vegetative cells present in a given sample of soil. The method is based on the known fact that resting cells can withstand conditions of desiccation that are fatal to vegetative cells. It consists in counting the total number of individuals in one part of a well-mixed freshly gathered soil sample by means of a cultural dilution method, and in counting the number of resting or resistant forms in a second part of the same sample after it has been subjected to rapid desiccation by means of a stream of dried sterile air at laboratory temperature, the vessel containing the soil being immersed in a water bath at 35°C. so that the conditions should approximate to those of a dry wind on a hot day. A comparison of the two counts gives some idea of the proportion of vegetative cells to resting cells. It has been found that the different species do not all react in the same manner to this treatment but that their resistance to desiccation is very varied: diatoms appear to be entirely killed off and are therefore presumably all present in the soil in the vegetative condition; blue-green algae, except for one small sheathless species of *Oscillatoria* which was completely killed off, appear to be unaffected by the treatment, while the unicellular green forms reacted quite specifically. In one particular sample, *Pleurococcus vulgaris* Menegh, the most resistant species, was reduced only to rather more than one-third, while *Chlorococcum humicola* was reduced to one-twelfth of its numbers. In *Chlorella* sp. and *Heterococcus viridis* more than ninety-nine out of every hundred cells were killed off, and in *Bumilleria exilis* a few cells only survived. In fact, of a total algal population of about eighty thousand individuals per gram of soil, only about nine thousand survived after desiccation. This definite proof that the soil algae are actually present in large numbers in the vegetative condition raises their status as members of the soil population to a position not far short in importance of that of the other groups of soil organisms, the numbers of individuals in the Rothamsted soils being quite comparable with those of the protozoa.

### CARBON NUTRITION OF SOIL ALGAE

As stated above, since these organisms have been shown to grow vegetatively in the lower layers of the soil in complete darkness, the question of their carbon nutrition is of first interest; but although an extensive literature is gradually being built up on the nutrition of many of the lower algae which has been quoted freely in relation to the soil algae, yet our knowledge of the physiology of algal species actually isolated from soil is still very meager. Beijerinck isolated *Chlorella vulgaris* from garden soil and showed it to be enormously benefited by the presence of organic compounds in the culture media and to be capable of growing in complete



darkness for more than a year. Since that time the same species has been isolated by a number of workers from a variety of other habitats and its physiology has been studied from different points of view, and Beijerinck's observations have been confirmed and amplified, especially in regard to the alga's independence of light if given a suitable supply of organic food. Chodat has found, however, that three strains of this species which he has isolated from separate localities, while agreeing in their general characters, differ from one another in their finer physiological reactions sufficiently to justify their being regarded as independent varieties of the species: he therefore suggests that certain of the algae identified with Beijerinck's soil species are probably different strains of the species, since the identifications were made usually on a solely morphological basis, and there is no certainty that physiological facts established for other strains are necessarily applicable to the soil form.

Charpentier carried out an extensive investigation of the physiology of a unicellular alga which he isolated from soil and identified as *Cystococcus humicola*, Naeg. He found the alga to be independent of light in relation to carbon nutrition and to its use of certain nitrogen compounds; but he showed that growth in the dark was always very much less than that in the light under comparable conditions.

Recently Muenscher has obtained strong evidence that a large species of *Chlorella*, originally isolated from soil, can synthesize proteins in total darkness when carbohydrate is present and nitrogen is supplied in inorganic combination.

Apart from these species the investigation of the carbon nutrition of the lower semisaprophytic algae has been mainly directed towards algae isolated from other habitats, such as lichen gonidia or the algae of water polluted with organic matter; and although these observations are interesting in a general way there is little reason to believe, in my experience, that the species or varieties isolated from soil will necessarily be identical physiologically with those isolated from different habitats.

A recent comparison of the growth of five unicellular green algae, isolated from the same soil sample, on a number of media containing various organic compounds, both in diffuse daylight and in total darkness, showed that though all five species were capable of growing in the dark on some or all of the media, yet the responses of the algae to the conditions imposed on them were very different, not only in regard to the range of compounds they could assimilate in the dark but in regard to the amount of substance they could produce and the duration of the growth period. It is therefore quite wrong, in my opinion, to regard the soil algae as a homogeneous physiological unit, or to argue from one species to another in discussing the relation which these organisms bear to the problems of soil fertility; still less is it justifiable to apply to the soil algae facts which have been ascertained or theories which have been built up in connection with

organisms isolated in pure culture from quite other habitats, until it has been definitely proved that the organisms in the two cases are identical.

Apart from Chodat's observations in regard to the numbers of different strains of *Chlorella vulgaris* which exist in different localities, the following observation of the writer will serve as a striking illustration of the dangers involved in making general statements based on superficial resemblances of organisms to one another. Two pure cultures of soil algae, Nos. 6 and 11, were isolated from the same sample of soil, and were regarded for two years as duplicate cultures of the same organism. The microscopic appearance of the cells and the form and color of the colonies of the two organisms on the usual range of sugary media were sufficiently alike to justify this conclusion. When, however, the two forms were grown as shake cultures in gelatine media a difference in physiological reaction soon became apparent, for whereas No. 11 slowly but completely liquefied the medium as it developed, forming eventually a continuous green stratum which gradually sank within the medium as the underlying gelatine became attacked and liquefied, No. 6 grew in isolated pinhead colonies, and even at the end of twelve months there was no sign of liquefaction of the gelatine. Obviously the relation of these two strains to soil fertility is likely to be very different, and I would strongly emphasize the necessity for accumulating physiological data of this kind before any attempt is made to generalize in so complex a subject.

Considerable stress has been laid upon the saprophytic aspect of the nutrition of the soil algae since it is only by these means that the algae can establish themselves as true members of the soil population, but it is by no means certain that this is the only, or even necessarily the chief, aspect to be considered in regard to the relation which these organisms bear to questions of soil fertility. It has been shown that all the algae so far isolated from soil are capable of growth in complete darkness in the presence of suitable organic food, but it is equally true that in the light they are all able to grow healthily when they are deprived of all organic substances, and are obliged to build up their own organic food from carbon dioxide, water and mineral salts by photosynthesis. The question therefore arises as to the relative importance of these two types of nutrition when the algae are growing on the surface of the soil; are the algae then growing by photosynthesis and so enriching the soil in organic matter, or has the saprophytic habit so firmly taken hold that they continue to draw upon the existing energy sources of the soil and thus impoverish it? A general statement on this subject is not possible at present, but a series of quantitative physiological experiments carried out on a single species, *Scenedesmus costulatus* Chod, var. *chlorelloides* Bristol-Roach, shows that for this species at least the photosynthetic habit is the dominant one; for under conditions of light that are entirely favorable to photosynthesis there is apparently a definite maximum rate of growth which the alga has

never been observed to pass and which can be attained under purely photosynthetic conditions, and under these conditions the addition of glucose to the medium has no power to increase the rate of growth of the alga. Under lower intensities of illumination the rate of growth by photosynthesis decreases, but the deficiency may be made good by the absorption of glucose, if present in the medium, though the rate of growth never exceeds the maximum already mentioned. As the illumination is still further diminished a point is reached when the decrease in photosynthesis is no longer completely made good by the absorption of glucose; the rate of growth then progressively diminishes until darkness is reached, when growth is entirely dependent on absorption of glucose and the rate is about half of the maximum possible. It is hoped that full details of this work will shortly be published, but it is evident that on the surface of the soil the alga, given a suitable light intensity, will add considerably to the organic content of the soil and will only gradually adopt the saprophytic habit as conditions become unsuitable for photosynthesis. The adoption of the saprophytic habit however, will ensure that there are always available within the soil a number of vegetative cells which can rapidly resume growth by photosynthesis when the conditions again become favorable. It is not to be supposed that this relationship necessarily holds for all species of soil algae, for the species in question does not respond in diffuse light to the presence of sugars in the medium as much as certain other species under observation, e.g. *Cystococcus* sp., in which the saprophytic tendency may be more highly developed; *Chlorella vulgaris* Beij. also has been shown by Grintzesco to be capable of more vigorous growth in complete darkness given a suitable medium than in the light. But *Scenedesmus costulatus* is the only form which up to the present has been subjected to really critical experiment and for which therefore definite knowledge is available, and it is probably typical in behavior of at least a group of the soil algae, and until such work is supplemented in regard to other species it will be unwise to make general statements on this subject.

### NITROGEN CYCLE IN THE SOIL

Second only in interest to the carbon nutrition of the soil algae is their relation to the nitrogen cycle in the soil; this has received more attention than any other aspect of their physiological activities, especially in regard to their connection with the fixation of atmospheric nitrogen. The literature up to 1922 has been critically reviewed in experimental papers on the subject by Schramm and by Bristol and Page, and the weight of evidence is wholly against the fixation of nitrogen by algae alone; for all the experiments which have lent color to the theory that algae fix nitrogen have been shown to be unreliable, either on account of the presence of other organisms in the cultures or on account of faulty chemical technique, whereas the really reliable experiments have invariably discountenanced

the possibility. The work of Wann, published in 1921, which was most carefully carried out on the biological side with pure cultures of algae mainly derived from soil was found to be subject to serious errors from defective chemical methods by Bristol and Page, who failed to corroborate his results, working as nearly as possible under identical conditions; rather they obtained some evidence of a slight loss of nitrogen from the best grown cultures. Further, Muenscher working with a large species of *Chlorella* and the writer, working in collaboration with G. C. Sawyer late of the Chemical Department, with *Scenedesmus costulatus* var., have failed to obtain any evidence of fixation by these two algae in pure cultures in liquid media, a result already obtained by Charpentier for *Cystococcus humicola* twenty years earlier.

On the other hand, evidence is increasing that the algae probably do play a part, though an indirect one, in nitrogen fixation under natural conditions; for all the work that has been published on the question from Kossowitsch to Lipman and Teakle indicates that the nitrogen fixing bacteria show an increase in the amount of nitrogen fixed when algae are present in the culture. It has been shown that nitrogen fixing bacteria only remain active so long as the products of their metabolism are constantly removed from their sphere of action, and it may be that the stimulating effect of the algae may be due to their using up the nitrogen compounds as soon as the bacteria produce them; or the algae in their mucilaginous sheaths may contribute, as has often been suggested, readily available carbohydrate as an energy supply to the bacteria.

Evidence in regard to the condition in which the soil algae absorb their nitrogen is very scarce. Experiments at Rothamsted on four different species showed that in media containing mineral salts alone ammonium sulfate produced a better growth of all four than an equivalent amount of nitrogen present either as ammonium nitrate or as calcium nitrate; in the presence of glucose, on the contrary, ammonium sulfate was most unsatisfactory for all species, two of them growing most luxuriantly with calcium nitrate while the other two grew best with ammonium nitrate.

Muenscher has found that a large species of *Chlorella* from soil was able to use nitrogen in the form both of calcium nitrate and of ammonium sulfate for the synthesis of protein in light of moderate intensity and also in complete darkness, but that at the end of 106 days the yield of algae in the calcium nitrate medium was very much in excess of that in the ammonium sulfate medium, suggesting that for this species ammonia may be a less suitable source of nitrogen than is nitrate. This is further borne out by the fact that the nitrate cultures gave approximately the same yield of algae in the light and in the dark, whereas the alga grew less well in the dark than in the light when the nitrogen was present as ammonium sulfate. In both series of cultures the nitrogen content of the algal crop was lower in the dark than in the light.

Unpublished data in regard to the daily rate of uptake of nitric nitrogen by *Scenedesmus costulatus* var. in diffuse light, obtained in collaboration with G. C. Sawyer at Rothamsted, show that during the period of vigorous multiplication of the alga, lasting 9 or 10 days, the amount of nitrate absorbed from the medium was directly proportional to the bulk of alga produced, but that during the later stages of the cultures, when the rate of increase of the alga was becoming progressively slower, the amount of nitrate absorbed per unit bulk of alga progressively diminished until towards the end of the experiments the nitrate content of the medium remained fairly constant at about one-third of its original strength. At this period, though growth was still taking place in the cultures there was at the same time death and disintegration of a proportion of the cells, and it is possible that the nitrogen compounds set free in this way may have been replacing the directly available nitrate as the source of nitrogen to the multiplying cells. On the other hand, the evidence showed that there was a decrease in nitrogen content of the alga towards the end of the experiments.

The ability of the soil algae to use protein as a source of nitrogen has been demonstrated by the fact that ten species tested in pure culture all produced considerable growth on a medium consisting of distilled water with 10 per cent pure gelatine, though two of the species grew conspicuously less well than the rest. At the other extreme, four species grew luxuriantly and completely liquefied the medium in 16 days or longer.

### SUMMARY

In attempting to sum up our present knowledge or to formulate a theory in regard to the possible rôle played by algae in the soil, one is therefore faced in all quarters by the fact of the extreme variability of the physiological reactions of the different species to the conditions that have been imposed upon them, in regard to both the carbon and the nitrogen cycles in the soil. It appears from the evidence available to be quite unjustifiable to regard the soil algae as a homogeneous physiological unit, and much more physiological work is needed before it can be determined to what extent and in what direction the algae may supplement the activities of bacteria and fungi in soil, or whether, on the contrary, their chief importance may lie in their ability under suitable conditions of light and moisture to add considerable quantities of organic matter to the soil.

# PRESENT AND FUTURE STUDIES OF SOIL FUNGI

C. THOM

*U. S. Department of Agriculture, U. S. A.*

## INTRODUCTION

It has long been recognized that any particular soil is a composite of inorganic materials and organic agencies in which fungi are but one of several major biological groups influencing both the chemical composition and the physical conditions encountered. Waksman, in 1916, cited 125 papers consulted by him in studying a series of soil fungi from New Jersey and the intervening ten years have been exceedingly fruitful in such investigations. Any attempt, therefore, to review paper by paper the contributions of this series of workers would leave very little time or space to present the particular phases of this subject which have impressed me. Similarly a consideration species by species of the forms reported would leave no time for general considerations. Only a glance over such a bibliography is needed to show that these investigations are predominantly biochemical, that is, concerned more with the chemical conditions encountered in the soil as a result of the various activities represented, than with the soil as an environment for microorganisms and concerned with selected species as a basis for quantitative laboratory experiment rather than as active elements in a soil population.

Among these reports, investigations of filamentous fungi take one form as floras from representative soils of a series of widely separated regions in Europe, and such American states as New York, New Jersey, the District of Columbia, Michigan, Iowa, Georgia, Texas, Louisiana, Colorado and California, and a second form as intensive biochemical studies (*in vitro*) of selected species. In surveying the work already done in America, we may find suggestions for the arrangement and classification of the data accumulated as a basis for a constructive program for future studies.

## ENUMERATION OF SOIL FUNGI

At the outset in considering these floristic studies, the enumeration of soil fungi presents many difficulties. Field collection and objective enumeration can be applied only to the coarser forms such as the *Hymenomyces* and the fleshy *Ascomycetes*. Even with these only the fruit bodies are recognizable and tell little of the extent and function of the vegetative mycelia which alone are significant to the student of soil prob-

lems. The so-called "molds" with their microscopic size and evanescent mycelia require special methods and equipment for demonstration because very few of them give any direct and specific indication of their presence and function to the student of physical conditions in the field. Agarics in the form of collections of fruit bodies constitute many great herbaria and form the subject of extensive monographs. Mushrooms have received intensive study as a crop by botanists, but their reflex effect upon fertility as they occur uncontrolled in nature has received little attention. In contrast, fairly elaborate procedures have been devised for studying soil molds and their activities either by the direct microscopic method of counting mycelia and fruiting bodies, or by quantitative dilution cultures designed to secure an estimate of the numbers of individual organisms and the identification in culture of the species present. The findings of both methods are regarded by Waksman and Skinner (27, p. 69) as showing a "marked parallelism" and as "giving a reliable index of the abundance of fungi in the soil," since they parallel in a general way the results of biochemical studies of the same soil. Both methods are, however, subject to serious limitations.

### MICROSCOPIC METHODS

Microscopic methods of counting spores, fruiting bodies, masses or fragments of mycelium have been developed and standardized by Conn, Waksman and Winogradsky. The value of these studies is strictly limited by the common impossibility of identifying the species represented in the fragments found. This is due partly to the intimacy of relation between the soil particle and the active fungus hypha. In our own soil inoculations with known species of *Aspergillus* and *Penicillium*, the hypha actively vegetating in the soil was found so adherent to successive particles that separation was impossible. Under extremely careful methods of preparation an occasional hypha was sufficiently isolated for observation and found so adherent to a series of particles that when straightened it held those particles together in a chain much resembling the relations between the unicellular root hair of the higher plants and the soil granules (Church and Thom). When such a chain was broken, however, the delicate fragments remained so intimately attached to the granules that their presence was not discernible to the user of the microscope, although many of these fragments grew readily when transferred to culture media. As an additional difficulty in the interpretation of microscopic findings, the mycelia and fruiting bodies of species recognizable in laboratory media by particular morphology did not show the same structures when found in soil. Microscopic examination can only be regarded as having a confirmatory relation to the findings of qualitative methods which provide means of identifying the species involved.

## CULTURE METHODS

In dilution for culture the comminuted sample of the microscopist is transferred to nutrient media, then after incubation the colonies of fungi are studied. The advantage of culture lies in ability to identify many of the species found. Its limitation consists in the complete failure of certain species to grow in our laboratory media and the failure of many other forms to produce the fruiting masses necessary for their identification. Culture upon a series of media of varying composition may accomplish much, but there is always danger of an arbitrary and distorted conception of the fungi of the soil since the findings often degenerate into total numbers of colonies calculated on dilution plates and separate lists of organisms identified without measuring the significance of the species involved. Again the forms growing rapidly commonly overgrow or completely suppress the species which grow more slowly there, hence the rapidly growing forms occupy a prominent place in our species lists without necessarily indicating that they actually hold so important a place in the soil complex.

In dealing with the results of both methods of enumeration, it must be remembered that the figures are strictly artificial. Although they are commonly reported as numbers of fungi in the soil, they are not numbers of individuals or entities comparable to bacteria, but numbers of viable cells and cell complexes, that is, fragments of fungus bodies, remaining in the sample after it has passed through a specific method of dilution, shaking, grinding, stirring or other preparation, whereas in actual fact the active fungi in the soil mass must be conceived of as a series of interlacing networks of the hyphae composing the mycelium of the various species, some of which as single mycelia may penetrate enormous masses of the substratum.

Figures resulting from breaking such soil masses into their finest particles and counting the fungous fragments found or the colonies growing from them upon media, have comparative value only to the extent that the methods of preparation are so standardized as to insure similar fragmentation of the fungus bodies occurring in successive samples. In the standardized procedures of a particular laboratory group, therefore, these counts may readily correlate with other studies of the same soils in giving a measure of the intensity of fungous activities.

## FUNCTIONAL CLASSIFICATION OF SOIL FUNGI

Many series of such cultural studies have been brought together by various investigators and the species found have been listed. When such lists are studied from the standpoint of the function of the individual species, four groups of species may be arranged as a basis for discussion.

(1) Species present but not actively growing, i.e., as spores, sclerotia, or other resistant forms.



(2) Species locally or occasionally active and significant.

(3) Plant parasites also capable of actively growing as saprophytes in the soil for considerable period at least.

(4) Endemic soil fungi.

While such groups necessarily overlap, they furnish a basis for analyzing the function of fungi found in the soil on the basis of their biochemical or economic importance even though some species might be readily placed in more than one of the groups.

#### GROUP 1

The earth as the general dumping ground of all organic debris receives every species of fungi in existence in vegetative conditions, as sclerotia, as spores or in all stages. The mummies of brown rotted fruits may be cited as representative of an important parasitic group found for a period in the soil, but which has only minor significance in studying soil problems. Some vegetative forms die with their hosts, and leave viable spores inactive in or on the earth as a source of future infections. These spores persist for a longer or shorter time and occasionally appear in our cultural studies as sterile or doubtful forms without giving tangible clues to their source or significance. However important some of them may be from the standpoint of crop pathology, most of these names only clog our lists of soil fungi with numbers and names which have no real bearing upon fertility. It is probably safe to add that the industrious floristic student could probably obtain in this way all the species in the "Sylloge" if he cared to work sufficiently extensively and intensively and for a long enough period of time.

#### GROUP 2

The exceedingly varied character of the soil presents local situations in which particular chemical and physical conditions favor the development of special organisms while conversely the species themselves may be active agents in the maintenance of these conditions. In this way certain forms become locally dominant hence determining factors in maintaining availability of that soil for particular purposes. Specific investigations of these special cases are desirable and important for growing special crops, but should be used with caution, in making general conclusions as to the broader problems of fertility. The very narrow limits to the distribution of certain of the *Hymenomycetes* together with their persistence within such areas is the typical illustration of this type of soil fungus well-known as an observation but resulting from causes very poorly understood.

#### GROUP 3

*Parasites:* Among the forms constantly found are some groups of species which produce destructive diseases to special crops, yet are capable of

maintaining themselves in the soil either permanently or for such periods as to imperil successive crops of the host species in the areas infected. As conspicuous instances of such infection the flax-sick soils studied by Bolley, the widespread occurrence of the scab organism of potato (*Actinomyces scabies*) which has been studied by a succession of such workers as Thaxter, Jones, Lutman, the whole series of *Fusarium* diseases which infect such varied crops as tomatoes, egg plant, cotton, bananas, etc., the destructive effects of species of *Pythium*, *Rhizoctonia*, *Ozonium*, and among the Agarics the attacks of *Armillaria* upon tree roots, of *Marasmius* in the fields of cane. All of these organisms are believed to be actively growing in the soil and to be agents in the decomposition of organic matter hence factors in producing plant food, however useless the infected soil with its added fertility may be found for the growing of particular host species.

#### GROUP 4

*Endemic soil fungi:* The existence of a flora specific to the soil has been questioned. If we compare the species gathered by widely separated workers, however, we find among them certain forms which occur with minor variations throughout all these studies. Our own studies have covered the organisms collected by Miss Dale in England, by McBeth and Scales in various fields, including California, Georgia, Virginia and Maryland, by Werkenthin in Texas, by Pratt and Zundel in the potato fields of Idaho, by Hartley in coniferous seed beds in Kansas, by Jensen at Ithaca, New York, by workers at Providence, R. I., and in addition to hundreds of incidental cultures sent in by collaborators, our own collections have included organisms personally taken from many hundreds of petri dish cultures made by Esten and Mason at Storrs, Connecticut, personal collections in Kansas, Illinois, Louisiana, and numerous isolations from dirt contaminated substances of widely separated origin.

In all these series, the cosmopolitan saprophytic genera, *Penicillium*, *Aspergillus*, *Fusarium*, *Trichoderma*, and the *Mucors*, form the vast majority of the cultures occurring. Roughly, it is perhaps safe to estimate that the *Mucors*, the *Aspergilli* and the *Penicillia* will form half or more than half of the actual cultures found in such platings. In our own culture work, as in the materials received from others, we have the same floristic picture that is reflected in the papers of Hagem, Traaen, Lindner, Dale, Oudemans, Waksman, and many others. *Mucors*, species of *Aspergillus*, *Penicillium*, *Fusarium*, *Cladosporium*, *Alternaria*, *Trichoderma* and associated genera, that is, the so-called "common" molds form the vast majority of the determinable species encountered. In spite of the occasional unique form among these groups, the molds as an aggregate are cosmopolitan and saprophytic, capable of growing upon a wide range of organic materials and under varied conditions of temperature, moisture, and oxygen tension.

Comparing the lists of molds encountered commonly in our own field (forage and food mycology) with these soil studies, confirms the conclusion of Waksman, that certain of these groups are very widely present in field soils and among growing crops; for example, species of the genus *Zygorhynchus* and many others of the *Mucors*, the multitude of varieties of *Aspergillus fumigatus*, *A. nidulans* and *A. terreus*, certain species of *Penicillium*, *Rhizoctonia* and *Fusarium*, and the *Actinomycetes*.

Particular species among these groups have been subjected to intensive study. Hopffe selecting a strain of *Aspergillus fumigatus* found it so active in decomposing cellulose that she named it *A. cellulosae*. Waksman in a recent paper gives an intensive study of a *Penicillium* isolated from New Jersey soil and found a rapid destroyer of cellulose. This strain is a member of a group found by us (see Pratt) in every soil series investigated and so characteristic of soil studies as to justify the group designation "soil penicillia."

Similar investigations will undoubtedly show many forms capable of participating in the reduction of plant and animal remains toward the simpler compounds favorable for the metabolism of the green plants, but these experimental results must be carried to the field and tested under field conditions before we can feel that we have made real progress. As indicated already, however, there is a notable lack of information as to the potential activities or the actual part played by the mycelia of the higher fungi in these processes. Thom and Lathrop working with bagasse showed that the mycelia of such Agarics as *Psilocybe* were capable of penetrating for long distances into enormous masses of organic debris and producing notable changes, with great rapidity. The field student of the *Hymenomycetes*, finds certain series such as species of *Agaricus* (*Psalliota*), *Psilocybe*, *Pholiota*, *Galera*, *Naucoria*, *Hypholoma*, *Hebeloma*, *Coprinus*, *Stropharia*, *Cortinarius*, and the *Lycoperdaceae* to be exceedingly common in our cultivated fields. During the summer of 1926, in sandy soil in Maryland during an intensely humid period, many hundreds of a single *Agaric* were found upon plant remains which were at or just below the surface of the soil and under conditions clearly indicating that the mushroom was a principal factor in the decay of this vegetation. Such Agarics produce caps only in favorable periods, but there is abundant evidence that their mycelia remain for long periods as active agents in producing decomposition. For example, I have found *Agaricus Rodmani* in the same spot along a street in Washington at two periods, spring and fall, when temperature and moisture conditions reach a particular point each year for 10 or more years. Similar instances will doubtless occur to anyone who has studied the Agarics for many years.

## THE SOIL AS AN ENVIRONMENT FOR ORGANISMS

These considerations bring us back to a discussion of the soil as an environment for organisms. Here as always the limiting factors in organic activity are nutrients, moisture, acidity, oxygen supply, temperature, and the presence or absence of specific inhibiting agents. Stated in another way, we need to measure the demands made upon the soil by the various groups of organisms encountered in our investigation as well as to estimate the contributions they are capable of making toward the usefulness of the soil in which they grow, for our particular purposes.

To flourish in a competitive environment jointly occupied by many groups of organisms, a mold must find there not only the necessary nutrients but physiological conditions so specifically favorable to that species as to give it an advantage over other forms also present. Optima measured in pure culture under controlled conditions while contributory information, are not a complete test of the significance of a fungus as a member of a complex community. Cultural determination of the demands made by a mold upon its environment and the limits of its tolerance for heat or cold, concentration of nutrients, acidity, and anaerobiosis, are valuable, but all of these studies must be supplemented by the test of its actual ability to thrive under the actual stresses of soil conditions before it may be regarded as a significant factor in the practical problem of producing better conditions for crop production.

Molds are rarely merely negative consumers of nutrients already present; they are more frequently active agents through secreted enzymes in the production of changes unfavorable as well as favorable in their environment. For example, they are exceedingly active agents in the decomposition of the whole range of carbohydrates, and some of them are active producers of organic acid within the substratum. If that acid remains unneutralized in the substratum, conditions are quickly rendered unfavorable for many other organisms. Students of mold enzymes have shown that a series of strains within a genetic group (Currie and Thom, Oshima and Church) may vary tremendously in their relative quantitative production of particular metabolic products, hence some of these strains may be important agents in situations in which they would be destructive to other and closely related races and, perhaps, to the cropping systems of the agronomist.

We need then not only to study our organisms group by group in determining their capabilities for good or evil in our program of increasing the usefulness of a soil, but to obtain such information as whether an organism can thrive at the temperatures actually occurring in the soil, at the oxygen tension found at the depths at which the desirable changes must take place, or in the amounts of water available for vegetative activity.

Enough has been said to show the range of problems confronting us and

that our studies valuable as they have been, have only begun and must be extended to embrace the broadest type of physiological investigations of all of the groups of fungi encountered. Although not limited to the needs of the soil mycologist, the series of "Studies in the Physiology of the Fungi," by Duggar and his collaborators at the Missouri Botanical Garden represent an attack upon the whole problem of fungus activity. Such investigations need to pass beyond the laboratory and the pure culture and embrace the whole field of natural history of the species investigated, to add to the study of the organism in pure culture, extensive observation of its relations to its environment.

### SUMMARY

Our chemists have measured step by step the activities in progress in the soil by the progressive changes in the chemical combinations found in successive samples obtained under known conditions. Our biochemists have taken organism by organism and shown that certain of the species found in the soil produce enzymes capable of effecting part or all of these transformations. Lists of the enzymes produced by species of *Penicillium*, *Mucor*, or *Aspergillus* show them to be so nearly omnivorous that one intensive student of the *Aspergilli* declared that *Aspergillus niger* could be made to perform any enzymic feat the investigator wished, if he would only learn to put his organism under favorable conditions.

This then is the task still before us, to take the accumulating knowledge of the ends sought in the soil as the basis for crop production, and the accumulating knowledge of the active agents available and seek to make every agent contribute its maximum to an ideal of fertility. To state the problem in general terms is simple. To discriminate among the many tasks before us and select those which will lead us most surely toward real progress will tax our best judgment for a long time to come. For the immediate present the soil mycologist needs a better knowledge as to which fungi occurring in the soil are fundamental contributors toward profitable fertility and which are unprofitable "boarders" or even disturbers of the peaceful accomplishment of his purposes. In attaining that end, we must multiply our intensive studies of particular species not alone from the viewpoint of quantitative results of enzymic activity, but from the standpoint of practical usefulness as members of a competing soil population. Without losing sight of the quantitative measure of the usefulness of our organisms, we must broaden our knowledge to include the whole natural history of each group of the complex soil population.

### LITERATURE CITED

- (1) Abbott, E. V. 1926. A study of microbiological activities in some Louisiana soils. Louisiana Sta. Bul. 1924.
- (2) ———. 1926. Taxonomic studies on soil fungi. Iowa State College Jour. of Sci. 1: No. 1.

- (3) Bolley, H. L. 1913. Soil troubles and seed deteriorations. North Dakota Agr. Expt. Sta. Bul. 107.
- (4) Byars, L. P., and Gilbert, W. W. 1920. Soil disinfection with hot water to control the root knot nematode and parasitic soil fungi. U. S. Dept. Agr. Bul. 818.
- (5) Conn, H. J. 1918. A possible function of Actinomycetes in soil. N. Y. Agr. Expt. Sta. Tech. Bul. 64.
- (6) ———. 1922. A microscopic method for demonstrating fungi and Actinomycetes in soil. Soil Sci. 14: 149.
- (7) ———. 1918. The microscopic study of bacteria and fungi in soil. N. Y. (Geneva) Agr. Expt. Sta. Bul. 64.
- (8) Dale, E. 1912–14. On the fungi of the soil. I. Sandy soil. Ann. Mycol. 10: 452, 1912. II. Fungi from chalky soil, uncultivated mountain peat, and the black earth of the reclaimed finland. Ann. Mycol. 12: 33. 1914.
- (9) Duggar, B. M., and associates. 1916–26. Studies in the physiology of the fungi. Annals of the Missouri Botanical Garden. Published in numbered sections over the past 10 years.
- (10) Goddard, H. N. 1913. Can fungi living in agricultural soil assimilate free nitrogen? Bot. 56: 249.
- (11) Hagem, O. 1907. Untersuchungen uber Norwegische Mucorineen I. Vidensk. Selsk. I Math. Naturw. Klasse 7: 1.
- (12) Heukelekian, H., and Waksman, S. A. 1925. Carbon and nitrogen transformations in the decomposition of cellulose by filamentous fungi. Jour. Biol. Chem. 66: 323.
- (13) Jensen, C. N. 1912. Fungous flora of the soil. Cornell Agr. Expt. Sta. Bul. 315.
- (14) Jones, L. R. 1926. The relation of fungi to soil deterioration. Jour. Amer. Soc. Agron. 18: 150.
- (15) Manns, T. F. Fungi of flax-sick soil and flax seed. Thesis. North Dakota Agr. Expt. Sta.
- (16) McLean, H. C., and Wilson, G. W. 1914. Ammonification studies with soil fungi. New Jersey Agr. Expt. Sta. Bul. 270.
- (17) Oshima, K., and Church, M. B. 1923. Industrial mold enzymes. Jour. Ind. Eng. Chem. 15: 67.
- (18) Pratt, O. A. 1918. Soil fungi in relation to diseases of the Irish potato in southern Idaho. Jour. Agr. Res. [U. S.] 13: 73.
- (19) Scales, F. M. 1915. Some filamentous fungi tested for cellulose destroying power. Bot. Gaz. 60: 149.
- (20) Slagg, C. M. 1926. New and unusual diseases and injuries of tobacco. Scient. Agric. 6, No. 6, p. 193.
- (21) Traaen, A. E. 1914. Untersuchungen uber Bodenpil ze aus Norwegen. Nyt. Nagaz. Naturw. Christiania. Bd. 52, Pt. 1, 20.
- (22) Waksman, S. A. 1916. Do fungi actually live in the soil and produce mycelium? Science N. S. 44: 320.
- (23) ———. 1916. Soil fungi and their activities. Soil Sci. 2: 103.
- (24) ———. 1918. The importance of mold action in the soil. Ibid. 6: 137.
- (25) ———. 1919. Cultural studies of species of Actinomycetes. Ibid. 8: 71.
- (26) ———. 1922. A method for counting the number of fungi in the soil. Bact. 7: 339.
- (27) ———, and Skinner, C. E. 1926. Microorganisms concerned in the decomposition of cellulose in the soil. Jour. Bact. 12: 57.
- (28) Werkenthin, F. C. 1916. Fungus flora of Texas soils. Phytopathology 6: 241.
- (29) Winogradsky, S. 1924. La methode direct dans l'étude microbiologique du sol. Chemie et Indus. 11: 215.
- (30) ———. 1824. Subsequent papers in Compt. Rend. Acad. Sci. [Paris] 179: 367.

# THE QUANTITATIVE STUDY OF SOIL FUNGI

W. B. BRIERLEY, S. T. JEWSON AND M. BRIERLEY

*Rothamsted Experimental Station, England*

## INTRODUCTION

An investigation of the soil fungi has been in progress for some time at Rothamsted. The quantitative aspect only of the research will be considered here and the full paper upon which this summary of results is based, will appear in the *Annals of Applied Biology*.

No direct visual enumeration of fungi *in situ* is yet possible, nor is there any feasible method of extracting mycelium from soil by physical or chemical means. Further, any quantitative technique based on mass culture of soil suspension yields only a moiety of the fungi present. The numerous but slowly developing *Basidiomycetes* are almost deleted. Many *Ascomycetes* and *Fungi Imperfecti*, common in soil, but which germinate slowly or grow with difficulty on ordinary solid media are excluded, as are usually many *Phycomycetes* which require special methods of isolation. The number of kinds of fungi in the soil is legion and experience of soil mycology has led me to the considered opinion that there are perhaps few fungi capable of existing saprophytically which may not, sooner or later, be cultured from soil. Several authors have published lists of species that have been isolated but, if methods of selective isolation be applied over a considerable period of time it becomes obvious that these lists represent a fraction only of the number of fungi existing in a mycelial or a spore form in soil. The number of species that comes within the scope of any feasible quantitative method is very small, comprising only such forms as germinate and produce visible colonies on plates of mass suspensions within a period of ten days or so. If it were possible to synthesize in one estimation the several numbers of fungi isolable from any particular soil suspension by selective methods the total number of fungi per gram of soil sample obtained by the present technique would be multiplied manyfold. This procedure is not yet possible and an estimation of fungal content is based upon one or more mass extractions.

The present paper deals only with the quantitative technique of mass extraction, and the chief factors, as stated below will be considered seriatim: A. Sampling; B. Suspension; C. Disintegration; D. Dilution; E. Plating; F. Incubation; G. Enumeration.

## A. FACTORS OF SAMPLING

## I. METHODS OF SAMPLING

If sufficiently large samples are taken with due care no significant differences, greater than those occurring in replicate suspensions of single unmixed samples, are shown in fungal estimations of soil extracted laterally from a newly opened vertical soil face or collected by mechanical devices such as the screw auger, block sampler, etc. Choice of method is a question of suitability to type of soil and the nature of the experiment. Further, for all general purposes, until the primary suspension is made, the maintenance of strict aseptic precautions—beyond care and laboratory cleanliness—has not been found to make any significant difference to the results: from this step onwards asepsis is imperative and the slightest lapse may vitiate the experiment.

## II. AMOUNT OF SAMPLE

Examination of ordinary arable and grass-land and of the Rothamsted Broadbalk and Park grass plots has shown that, even in such presumably homogeneous soils as the two latter, the fungi may be distributed very unequally in closely adjacent areas. In Table 1 are shown the fungal numbers of eight replicate samples (A-H) taken at intervals of about one meter, one series on the unmanured Broadbalk III, two series on the farmyard manured Broadbalk II and one series on a local wheat field.

TABLE 1.—*Lateral distribution of soil fungi*  
(Dilution = 1/20,000)

Soil	Plate numbers from samples 1 meter apart							
	A	B	C	D	E	F	G	H
Broadbalk III	31.0 <sup>a</sup>	25.2	33.9	41.1	30.1	37.4	24.5	31.1
Broadbalk II	66.2	75.9	68.0	87.9	79.1	92.7	74.0	52.3
Broadbalk II	41.1	70.0	61.8	79.0	64.6	86.8	71.0	70.3
Arable field (wheat)	66.6	88.2	70.8	49.1	90.7	75.1	96.3	74.3

<sup>a</sup> Each number is the average of 8 plates.

Gross differences due to local "pocketing" resulting from horse droppings, manure patches and so forth are, as a rule, easily avoidable, but differences coming within the range shown in Table 1 are an integral feature of the fungal content of soils. To eliminate errors of selection it has been found desirable to take at least 6 samples each of 200–250 g. from the area to be tested; mixing these thoroughly and removing stones by passing the soil through a sieve. The heap is then divided vertically into



two, one-half again mixed and again divided and this process continued, 25g. of the final moiety being used for the making of the suspension. A second 25g. is weighed and dried at 100° C., to ascertain moisture content.

### III. DEPTH DISTRIBUTION OF FUNGI IN RELATION TO SAMPLING

In addition to sporadic lateral inequalities of distribution the soil fungi show a marked depth distribution. In Table 2 are seen the results of an examination of seven different soils, samples being obtained at various depths.

TABLE 2.—Depth distribution of soil fungi <sup>a</sup>

(Dilution = 1/20,000)

Kind of soil	Plate numbers at increasing depths in cm. <sup>b</sup>												
	0	2.5	10	15	20	25	30.5	45.5	61	91.5	122	152.5	183
A. Loam (tilled)	70.9	74.3	68.0	64.6	48.1		25.9						
B. Clay	18.1	33.4	25.7	12.1	7.0		9.3	0.6		0.5			0.2
C. Sandy common	20.6	27.1	28.7	30.7	15.4		5.1						
D. Broadbalk II	73.4	68.9	64.5	46.5	25.2	24.3	12.6	4.3					
E. Broadbalk III	53.3	47.4	51.2	27.5	22.6	18.7	13.3	6.2					
F. Parkgrass II	42.0	42.8	46.6	42.0	35.5		24.7	12.0					
G. Untouched loam	100.8	100.9	101.2	103.8	98.6		58.6	23.0	3.3	4.5	0.2	0.2	0.2

<sup>a</sup> A, B and C are comparable for fungal content, and also D, E and F.

<sup>b</sup> Each total is the average of 8 plates.

In most soils that have been examined there is a rapid decrease in fungal content, beginning at a depth of 10 to 20 cm., which is probably related to depth of ploughing and presence of clay subsoil. The top 10 cm., irrespective of whether the land is arable or permanent pasture, show only minor but occasionally significant numerical differences and these are eliminated for practical purposes if comparative samples are made up of soil representing adequately the top 10 cm.

### IV. STORAGE OF SAMPLES

It is often necessary to store soil samples until opportunity occurs for examination. The effects of storage differ according to the type of soil, the method of storage and the period involved but highly inconsistent results are often obtained even in replicate samples stored under, as far as possible, identical conditions. In Table 3 are shown the results of an experiment in which heavy allotment soil was thoroughly mixed and divided into three portions. One of these was kept on the allotment until the other two were air-dried. Each large sample was then divided into three and the experiment conducted in triplicate (A, B and C).

TABLE 3.—*Effects of soil storage on fungal count*  
(Dilution = 1/20,000)

Method of storage of heavy loam (H <sub>2</sub> O = 21.5)	Replicate Bottles	Storage time in weeks								
		0	1	2	3	4	12	24	36	48
Moist soil in large bottles with tightly fitting corks	A	39.3 <sup>a</sup>	54.7	60.5	103.9	180.0	?	?	?	?
	B	45.8	56.1	41.3	30.7	24.3	28.9	125.0	149.8	170.0
	C	43.1	69.0	45.0	30.8	28.2	67.5	43.1	?	110.2
Air-dried soil in large bottles with tightly fitting corks	A	54.7	43.3	39.8	35.5	37.3	25.2	30.7	22.4	20.2
	B	50.9	45.2	47.7	50.3	55.5	40.6	43.8	37.2	28.1
	C	55.3	58.4	60.2	73.8	75.4	53.5	57.3	50.5	59.0
Air-dried soil lying loosely on platters covered by bell jars	A	49.8	53.7	55.0	52.3	48.1	55.9	50.3	45.5	40.1
	B	56.5	50.2	49.1	55.8	60.3	48.5	46.7	42.3	49.8
	C	51.7	55.5	50.2	48.9	50.0	41.0	46.5	42.1	38.7

\* Each number is the average of 8 plates. ? = too many colonies to be counted, more than 200 per plate.

Relatively enormous variations are found in comparable samples stored moist in closed bottles, the variation being irregular but the numbers showing a tendency to increase during a year's retention. Thoroughly air-dried samples in closed bottles show smaller inconsistent variations with a general tendency for numbers to decrease during twelve months' storage. The most consistent results are obtained from air-dried soil maintained in a loose well aerated condition on platters covered by bell jars and kept in a cool place. Darkness or exposure to light does not appear to influence the results.

## V. COMPARISON OF SAMPLES

Even if errors of selection due to factors already mentioned have been avoided there is still the possibility that the fungi, like many other types of population, may show a seasonal periodicity so that the one soil, sampled at different times, may not give congruent results. During the earlier portion of this research the data then available seemed to show that a marked seasonal rhythm occurred in the fungal content of soils. Later work has not confirmed this although it has not denied the possibility. In ordinary soils the numbers, calculated on a moist or on a dry basis, seem to vary throughout the year about a general average level, with an indication of higher numbers in summer and lower numbers in winter. The average level is different for different soils or different manurial treatments on the one soil. Continued experience has shown that the numbers upon which the suggestion of seasonal periodicity was based fall within the range obtainable from replicate samples of adjacent areas of the same soil at any one time. Further, if the total fungal contents of different soils or any one soil, obtained during this investigation be plotted on a time scale, they do not fit any periodic curve but scatter about a mean value.

## B. FACTORS OF SUSPENSION

### I. SAMPLING OF SAMPLE FOR PRIMARY SUSPENSION

In the making of the primary soil suspension it is essential that the sample be adequately tested. The greater the amount of soil used, the less, theoretically, is the error of selection but, on the other hand, the more difficult are the manipulative processes. In the experiment, recorded in Table 4, various amounts of a thoroughly mixed sample were suspended in 250 cc. of water and the experiment carried out in triplicate (A, B and C).

TABLE 4.—Size of samples and error of selection  
(Dilution=1/20,000)

Replications of sample	Amount of samples in grams				
	1 gram	5 grams	10 grams	25 grams	50 grams
A	30.5 <sup>a</sup>	45.0	50.0	42.9	43.8
B	39.3	51.5	46.5	45.9	47.5
C	54.0	37.4	41.3	47.4	45.1

<sup>a</sup> Each number is the average of 8 plates.

Modifications of this experiment were conducted to test the best ratios of amount of sample to amount of liquid to volume of container and the highest and most concordant results were obtained with 25 g. of soil suspended in 250 g. of water in a vessel of one liter capacity. Replicate samples of less than 20 g. give, with decreasing amounts, an increasing lack of consistency whereas, samples of 50 g. and more, do not give increased accuracy to commensurate for the added difficulties of manipulation.

### II. SHAPE OF CONTAINER

Round, conical, cylindrical and other vessels were tested and the one finally adopted was a Roux culture bottle, flat type, of one liter size. This type of vessel is strong and easily obtainable, is of standardized shape, is convenient to hold or attach to a shaking machine and is of such a form that good "impact" of the suspension occurs on shaking.

### III. SUSPENSION LIQUID

In the making of the primary suspension, physiological saline was compared with distilled water and tap water but the results, an example of which is given in Table 5 show no significant differences.

TABLE 5.—Comparison of suspension liquids  
(Dilution = 1/20,000)

Duplicate series	Suspension liquid		
	Distilled water	Physiological saline	Tap water
A	44.9	39.0	41.1
B	47.5	43.9	46.8

#### IV. STANDING OF SUSPENSION

In the carrying out of replication or comparative experiments it is frequently necessary to prepare a series of shaken suspensions of which the earlier ones must be left standing until the last is completed. The effect of such standing of a suspension upon the final plate counts is shown in Table 6 the experiment being carried out in duplicate. (A and B).

TABLE 6.—Effect of standing of suspension on plate numbers  
(Dilution = 1/20,000)

Duplicate series	Time of standing of suspension in hours											
	0	1	2	3	4	6	8	12	18	24	48	72
A	46.1*	42.3	45.7	41.1	46.5	54.1	67.2	135.1	183.2	170.9	?	?
B	46.8	44.7	40.2	44.8	45.0	48.7	56.3	82.9	79.3	129.0	200.7	?

\* Each number is the average of 8 plates ? = more than 200 but exact numbers uncountable.

Standing for about four hours or less has been found to make no significant difference to the final number of colonies on the plates but after this period a variable increase usually takes place which rapidly destroys the value of the suspension.

### C. FACTORS OF DISINTEGRATION

#### I. METHOD OF DISINTEGRATION

Various methods of disintegrating the soil in the liquid and producing a fine and homogeneous suspension have been tested, such as stirring, grinding in a mortar, egg-whisking, air bubbling in various ways and shaking. The latter is by far the most convenient and satisfactory method, produces complete homogeneity of suspension, is easy to standardize and gives uniform results in replication tests. Various methods of shaking have been examined such as a mechanical wheel shaker driven by gas engine, a horizontal to-and-fro shaker driven by water turbine

and shaking by hand in various ways such as horizontal sliding to-and-fro on a bench, rotatory motion, vertical up-and-down motion and so forth. In all cases the speed was standardized by metronome. For experiments in which shaking for long periods is required a water turbine to-and-fro shaker gives good results. For all general purposes the best results are obtained by hand-shaking, the Roux bottle being shaken up-and-down over a distance of about 30 cm. in a slight arc, the elbow remaining comparatively steady and the speed being standardized by metronome at 120 per minute.

## II. VIOLENCE AND DURATION OF SHAKING

The spores and hyphae of fungi adhere to the soil particles and the degree of disintegration of the soil held in suspension largely determines the number and freedom of fungal moieties, each of which, when the final dilution is plated, may originate a colony. The fineness of disintegration depends upon the violence and duration of shaking, which, in turn, depend in some measure upon factors mentioned under the previous heading. The necessity of standardizing the shaking of the primary suspension and the general relations of fineness of disintegration to the number of colonies developing on the plates will be clear from the results given below.

Figure 1 shows a graph of the final plate numbers obtained from a light sandy-loam in which the primary suspension was shaken gently by a sliding horizontal motion over a distance of about 30 cm. on a bench at a metronome rate of 80 per minute. Samples were taken at the intervals noted and the colony numbers show a gradual and comparatively steady increase almost doubling during the two hours for which the experiment was performed.

Figure 2 shows a graph of the final plate numbers obtained from a compost heap of farmyard manure and loam in which the primary suspension received the most violent up-and-down shaking possible by hand at a metronome rate of 120 per minute. Samples were taken at the stated intervals. The colony numbers attain a maximum in 15 to 20 minutes and then remain comparatively steady for the remainder of the two hours of the experiment.

Figure 3 shows a graph of the final plate numbers obtained from a light dry loam in which the primary suspension was shaken from 10.15 A.M. until midnight on a water turbine to-and-fro shaking machine at a metronome rate of approximately 168 to 170 per minute. The machine gives a jerky motion over a horizontal distance of about 5 cm. and the actual violence of impact was, as far as could be judged, approximately intermediate between the gentle shaking shown in Fig. 1 and the extremely violent shaking shown in Fig. 2. The colony numbers attain a maximum in 45 to 75 minutes, remain relatively constant for about 6 hours and then

show a marked and steady decline which continues for the remainder of the period.

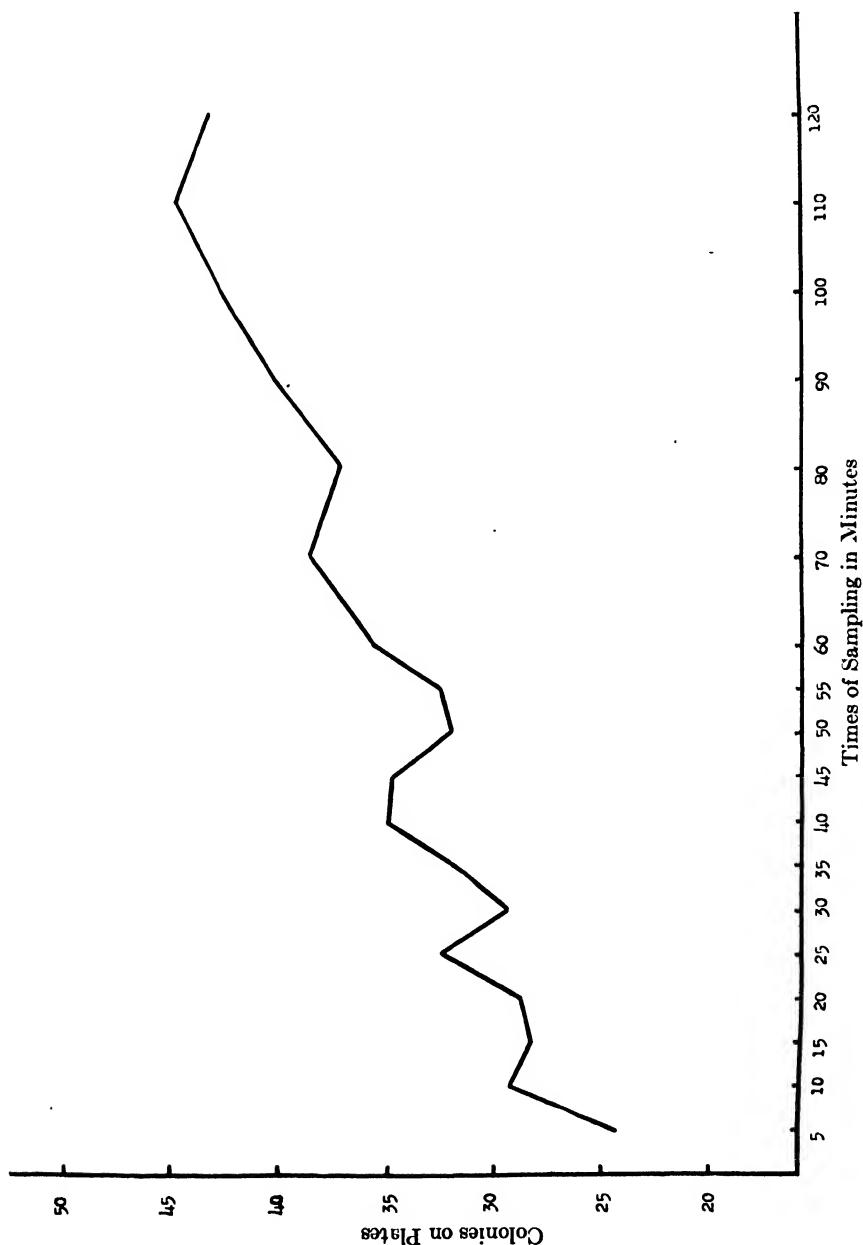


FIGURE 1.—Relation of violent shaking to plate numbers. Dilution  $1/20,000$

It is interesting to compare the decrease in colony numbers shown in Fig. 3 with the results obtained by submitting suspensions of pure cultures of fungi to prolonged shaking. Purely vegetative colonies of *Rhizopus*

*nigricans* and *Penicillium lilacinum* were macerated as finely as possible with needles and suspended severally with 25 g. of silver sand in 250 g.

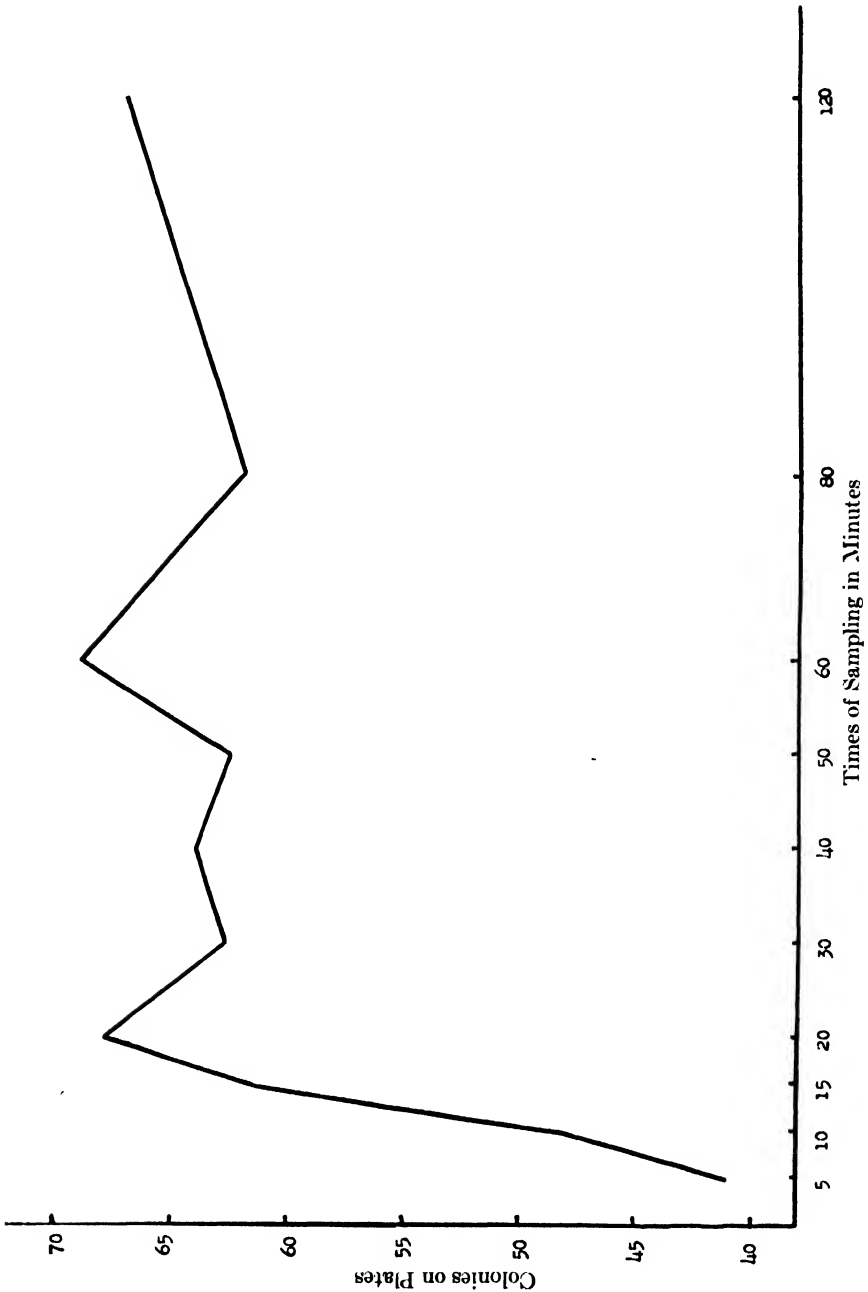


FIGURE 2.—Relation of violent shaking to plate numbers. Dilution 1/20,000

of water in a Roux bottle. They were then treated exactly as a primary soil suspension and shaken continuously on a turbine to-and-fro shaking

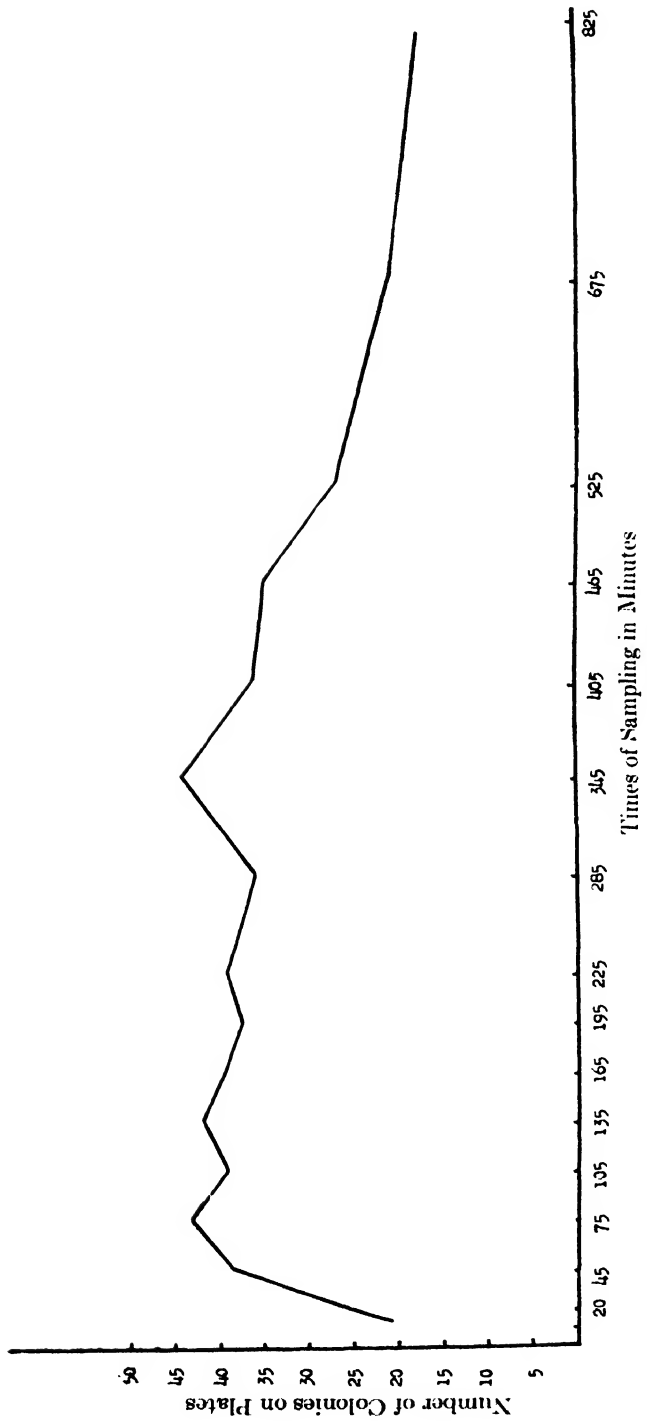


FIGURE 3.—Relation of moderately violent shaking to plate numbers. Dilution 1/20,000



machine at a metronome rate of 168 to 170 per minute, samples being taken at intervals. In a third experiment a suspension of spores only of *P. lilacinum* was treated similarly. The results are given in Table 7.

TABLE 7.—Effect of prolonged shaking on suspensions of pure cultures of fungi

Duration of shaking	Colonies on plates		
	Mycelium only <i>Rhizopus nigricans</i>	Mycelium only <i>Penicillium lilacinum</i>	Spores only <i>Penicillium lilacinum</i>
20 minutes	?	?	167.8
40 Do	123.1 <sup>a</sup>	?	170.1
1 hour	90.3	?	179.7
2 hours	37.5	190.5	164.8
4 Do	21.5	167.7	151.0
6 Do	14.9	130.0	130.5
8 Do	9.3	161.3	107.1
10 Do	15.1	188.1	98.0
12 Do	4.0	120.2	103.8
24 Do		27.9	52.4
34 Do		13.4	20.3

<sup>a</sup> Each number is the average of 8 plates. ?=more than 200 but exact numbers uncountable.

With certain fungi, more particularly the young mycelial stages of coenocytic forms, it is possible practically to sterilize a suspension of a pure culture by prolonged shaking with silver sand.

In the experiments recorded in Figs. 2 and 3 in which moderate and violent shaking of the suspensions occurred a large development of actinomycetes took place towards the end of the fungus developmental period. After the fungi had been counted the plates were examined for the actinomycete numbers and these were recorded separately and are shown in Figs. 4 and 5-A. It will be seen that, with shaking of moderate violence (Fig. 4), the numbers increase steadily but slowly, doubling during a period of 13½ hours. With violent shaking (Fig. 5-A) the numbers increase rapidly and steadily, quadrupling in a period of 2 hours and reaching the astonishing figure of approximately thirty-four million per gram of soil. Repetition of the "violent shaking" experiment using the same compost heap but at a dilution of 1/120,000 gave a similar result shown in Fig. 5-B.

In all general experimental work up-and-down hand shaking over a distance of about 30 cm. at a metronome rate of 120 per minute during a period of 20 minutes has been adopted and this has given high and very consistent results.

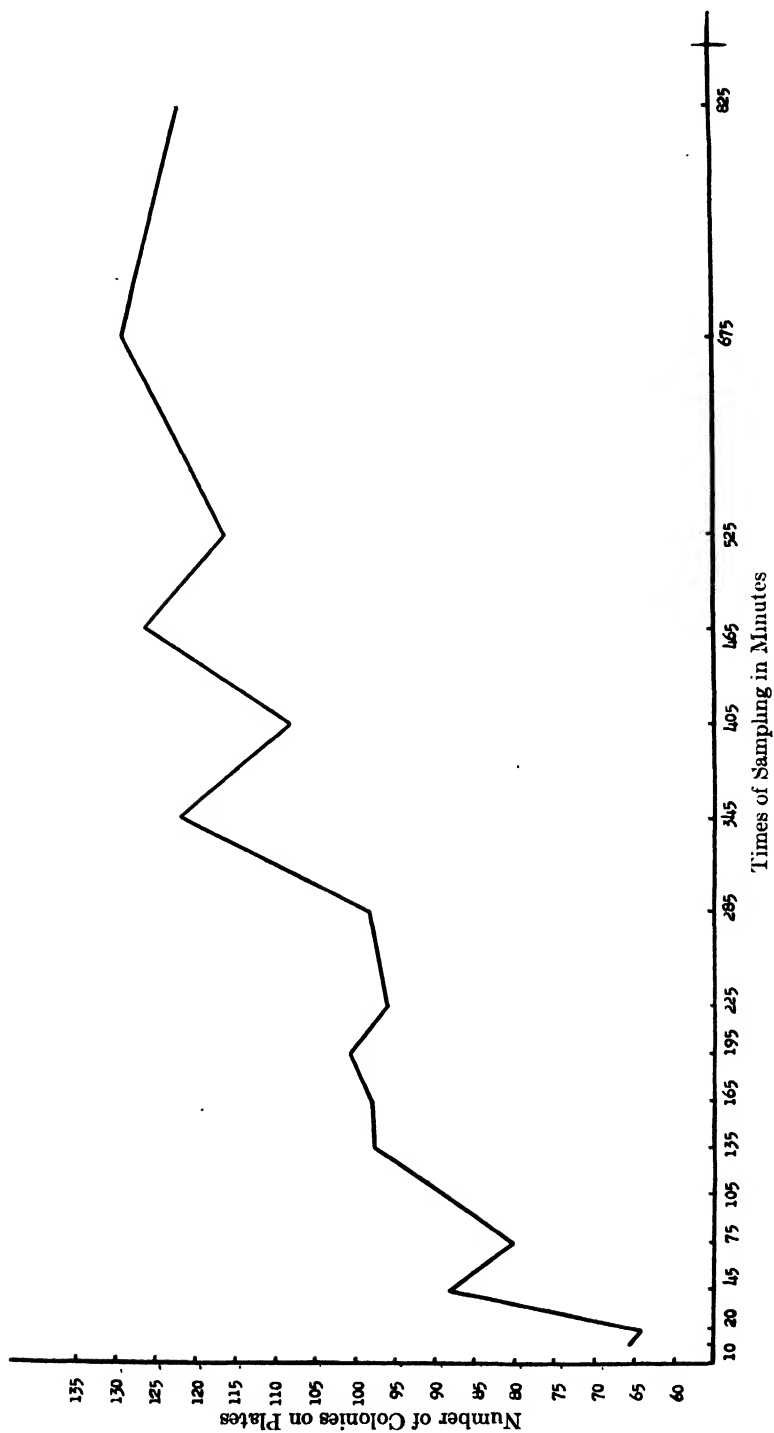


FIGURE 4.—Relation of moderately violent shaking to numbers of Actinomycetes on plates. Dilution 1/20,000

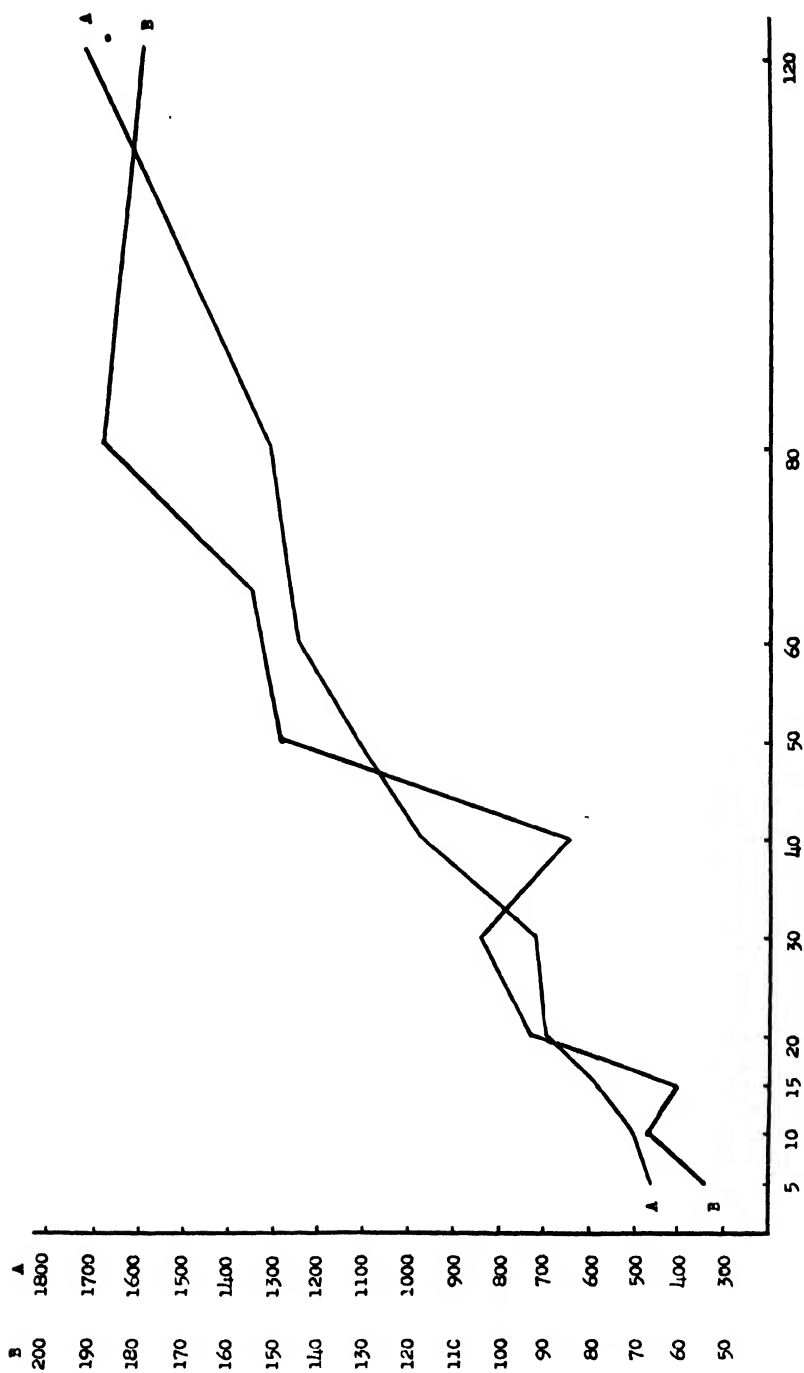


FIGURE 5.—Relation of violent shaking to numbers of Actinomycetes on plates. Dilution A = 1/20,000; B = 1/120,000

## D. FACTORS OF DILUTION

## I. METHOD OF DILUTION

The primary suspension of 1/10 requires diluting before it can be used as inoculum and from this stage onwards the most rigid precautions against contamination are imperative. In pipetting from the suspension into a prepared series of bottles containing the requisite amounts of sterile water, the utmost care is necessary that adequate samples of each dilution are obtained, which implies the occurrence of a homogeneous suspension at the moment of sampling. A considerable increase in accuracy and in congruence of results in replication experiments comes from experience and practice in pipetting. It is often necessary to plate parallel series of dilutions e. g. 1/10,000, 1/20,000, 1/40,000 in order to ascertain the most suitable for the particular soil sample or experiment but a dilution of 1/20,000 has been found to be the most generally desirable in practice. This is obtained as follows:

5 cc. of primary suspension (1/10) 45 cc. water = 1/100.

5 cc. of 1/100 dilution 45 cc. water = 1/1,000.

10 cc. of 1/1,000 dilution 190 cc. water = 1/20,000.

## II. DEGREE OF DILUTION

It is undesirable that single final plates contain more than 50 to 60 colonies and the best dilution to use is that which will give an average of 35 to 45 colonies per plate. Theoretically, a series of dilutions of one suspension, increasing in geometrical ratio should produce in the corresponding plates a series of numbers decreasing in geometrical ratio. In practice this is not the case and the numbers of fungi on plates of denser suspension are often considerably less than should occur theoretically as computed from the numbers of colonies on plates of high dilution. In Table 8 are shown the results of one such experiment.

TABLE 8.—Degree of dilution in relation to plate numbers

Dilution	1/2,500 8 plates	1/5,000 8 plates	1/10,000 8 plates	1/20,000 8 plates	1/40,000 10 plates	1/80,000 15 plates	1/160,000 15 plates
Numbers obtained	174	124	82	58	35	17	9
Theoretical numbers on 1/160,000 basis	576	288	144	72	36	18	9

If such a series of dilutions of one suspension be run in duplicate on the same medium a uniform rate of decrease in higher dilutions is found in each series. If corresponding series be run on different media the rates of decrease alter consistently with the medium. This is shown in Table 9 which gives the results of a comparative experiment on Waks-

man's medium and on Conn's medium, duplicate series (A and B) being run throughout. This table also indicates the unreliability of very high dilutions, although at times (Table 8) an astonishing accuracy is obtained.

TABLE 9.—*Degree of dilution in relation to rate of decrease in different media*

Dilution	Medium	1/2,500 8 plates	1/5,000 8 plates	1/10,000 8 plates	1/20,000 8 plates	1/40,000 10 plates	1/80,000 12 plates	1/160,000 15 plates
Numbers of colonies on plates	Waksman	A 61	51	35	20	12	6	7
		B 68	57	40	24	15	9	5
	Conn	A 114	76	50	36	21	10	4
		B 121	80	57	40	24	13	6

The rate of cutting down in a series of dilutions decreasing in geometrical ratio is a function of the particular kind of medium, and this is the case if the inoculum comprises a mixed soil population or, to a lesser degree, if a suspension of fungal spores from a pure culture be used. In the latter case it is also a function of the particular species, different fungi varying greatly in the rate of cutting down on a standard medium.

## E. FACTORS OF PLATING

I. *Method:* The larger the amount of dilution used as inoculum the less is the error of selection but the increasing uniformity of numbers theoretically resulting from the use of 2, 3, 4 and 5 cc. of dilution is more than offset by the practical difficulties introduced with increasing amounts such as rapid setting of medium with consequent lack of equal distribution of inoculum or, with certain media the lack of jelling. One of the most critical factors in plating is homogeneous distribution of inoculum for the slightest "clumping" interferes with colony development and often vitiates the plates. The most desirable ratio for use with plates of 9 cm. diameter has been found to be 1 cc. of dilution to 10 cc. of medium.

II. *Number of Plates:* The greater the number of plates from which an average can be obtained the more significant is that average. Comparative tests using 2, 4, 6, 8, 10, 12, 16 and 20 plates show that for all ordinary purposes 8 plates per series give a high degree of consistency. The slightly greater exactitude from more plates does not commensurate for the increased labor involved which, in large experiments, is a serious and may be inhibitive factor. Replicate series containing less than 6 plates often give unsatisfactory agreement and leave no margin for loss. Where higher dilutions producing lower plate numbers are used a correspondingly greater number of plates must be adopted, so that the total numbers of fungi compared per series, are approximately equal.

III. *Size of Plates:* To a certain extent the number of colonies on the plate is a function of the size of the plate, other things being equal. Greater isolation on larger plates appears to reduce the number of fungal

moieties that can form visible colonies, but, above a certain limit, the overcrowding on smaller plates also diminishes the number of colonies. In Table 10 is shown the type of result that is obtained when the same dilution is inoculated into plates of different size where the amount of medium per unit area of the plates is equal.

TABLE 10.—Size of plate in relation to number of colonies

Medium	Dia. of plate	Dilution			
		1/2,500 8 plates	1/10,000 8 plates	1/40,000 8 plates	1/160,000 8 plates
Conn	cm. 9	114±4	50±4	21±2	4±1
	23	143±7	34±5	12±2	5±1

The importance of the factor of plate size varies considerably with different media and it functions not only when the inoculum is a mixed population of soil fungi but, to a lesser extent, when suspensions of spores of pure cultures are used. In practice the inconvenience and expense of large plates renders their use impossible and, with all but very low dilutions, satisfactory results are obtained with small plates. The plates adopted throughout this work have been 9 cm. in diameter.

IV. *Amount of Medium:* The amount of medium has a slight and barely significant effect upon the number of colonies developing on the plates as is shown by the experiment recorded in Table 11 which was carried out in duplicate (A and B).

TABLE 11.—Amount of medium in relation to number of colonies  
(Dilution = 1/20,000)

Size of plate	Amount of medium	Number of colonies on plates	
		A 8 plates	B 8 plates
9 cm.	5 cc.	41±2	43±2
	10 cc.	33±2	35±2
	20 cc.	37±3	35±2

A similar result is also shown by plates of 15 cm. and 23 cm. in diameter the thinner layer of medium in each case giving the slightly greater number of colonies in duplicate series. The greater numbers from 5 cc. of medium in a plate of 9 cm. diameter are more than offset by the difficulty of obtaining a homogeneous distribution of inoculum and medium, owing to rapid cooling, and in occasional lack of jelling. In practice

10 cc. of medium per plate of 9 cm. diameter has been found to be the better amount.

*V. Composition of Medium:* The number of colonies that develop in plates depends to a certain extent upon the kinds and proportions of food stuffs available in the medium. If for example a single dilution be plated upon a number of commonly used media all brought to the same acidity the following type of result is obtained. In Table 12 the two columns refer to different experiments.

TABLE 12.—Composition of medium in relation to number of colonies  
(Dilution = 1/20,000)

Medium	Number of colonies *	
	pH = 4.1	pH = 5.6
Uschinsky	21	24
Prune	22	..
Cook	23	..
Waksman	24	31
Beerwort	25	35
Czapek	26	37
Coon	27	36
Thornton	27	38
Brown	28	36
Clay soil	30	43
Conn	35	49
Light loam soil	36	50
Rich manure compost	38	54

\* 8 plates average

Repetition of this experiment gives slight changes but the general order is maintained, Uschinsky's medium and Prune medium producing the lowest numbers and Conn's glycerine-sodium asparaginate medium and soil media the highest. Certain richer media such as Beerwort and Waksman's medium favor the rapid growth and reproduction of particular fungi and these tend to overrun the plates and produce secondary colonies at the expense of the more slowly growing forms. Before this happens, the colonies on these media are extremely clear and easy to count but once the plates are overrun they have little worth. Further, the value of a medium lies not only in the relative numbers of colonies that develop upon it but in the agreement of these numbers in replicate experiments. In this regard Conn's medium has given the most uniform results. Soil media give high numbers but the congruence of results in replication is not so good as upon Conn's medium, the colonies are extremely thin and diffuse and difficult to count, they show little characterization so that there is increased liability of confusion between fungi

and actinomycetes and the media cannot be standardized as can a synthetic chemical medium. For all general purposes Conn's medium has given the most satisfactory results, and no slight modifications of composition have been found to improve it.

VI. *Acidity of Medium*: Many investigations in soil mycology have had a dual aim, the examination of quantitative aspects being combined with floristic studies. In order to obtain easily, pure cultures of soil fungi from mass platings it is desirable to use a medium such as Beerwort, Richard's or Waksman's medium which gives clear, highly colored and characteristic growths. Such media, however, excellent as they are for floristic studies are most unsuitable for quantitative researches where the aim is not to produce a few vigorous and characteristic growths but to obtain the greatest number of discrete colonies possible per plate. Further, it is essential to eliminate, as far as possible, bacterial development and the most convenient way of doing this is to acidify the medium to a hydrogen ion concentration of about 4.0 at which point fungi develop and bacteria are inhibited. The common practice has been, therefore, to use a "floristic" medium at a high acidity. An acidity of a pH of 4.0, however, approaches the limit at which many fungi grow and is actually beyond the limit of certain forms and, therefore, if maximum fungal numbers only are required, the acidity is undesirably high. On the other hand if the medium approaches neutral point, bacterial growth is so great that fungal development is largely inhibited and at times almost eliminated. There obviously exists an intermediate range of acidity where a balance is struck between maximum fungal population and increasing bacterial development. The acidity for obtaining maximal fungal numbers has been ascertained by comparing series of plates inoculated with the same dilution, in which each series contained media of different acidity. A large number of such experiments have been carried out on the same and on different media the acidity values ranging from a pH of 3.7, which is the maximum for jelling to a pH of 6.6. The results are occasionally difficult to interpret, being at times highly inconsistent. In the majority of experiments the figures when plotted form a more or less smooth curve with a peak at a relatively fixed hydrogen ion value as shown in Fig. 6. In other experiments a rounded or flat topped curve has been obtained as shown in Fig. 7-A whereas in still other series the curve has been indeterminate (Fig. 7-B) as though some extraneous factor had cut across the acidity values.

The smooth curve with single peak has, however, been obtained sufficiently often in comparison with the more aberrant curves that it would appear to represent the true result. One must, however, recognize the fact that though the experiment be carried out with all critical precautions one cannot always rely on obtaining results fitting into this smooth simple curve. The numbers may be scattered about a mean



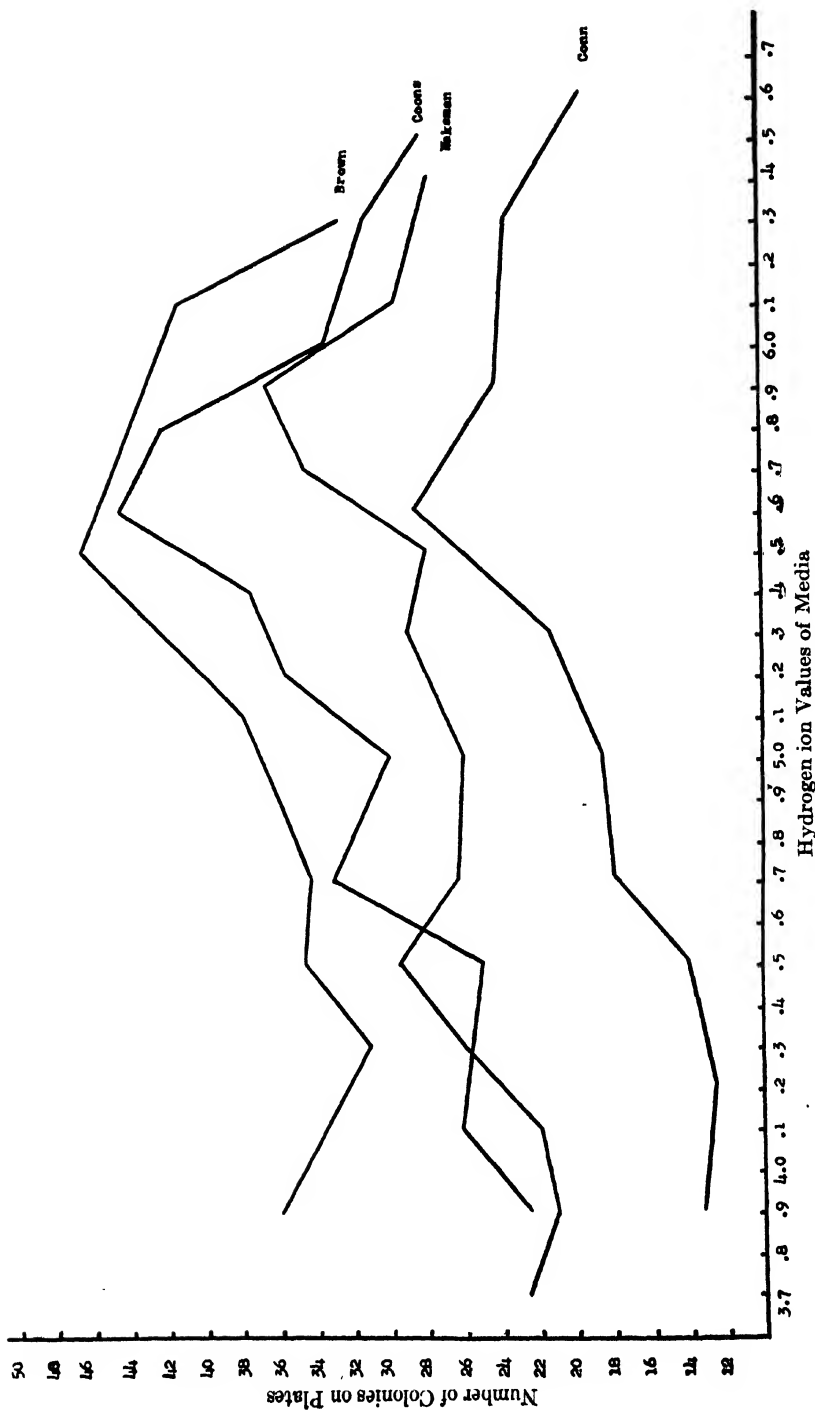


FIGURE 6.—Relation of acidity of medium to plate numbers. The results are from four different experiments and the curves are only comparable in shape. Each point is the average of 8 plates except the Conn series in which the points are the average of 10 plates and in each experiment the dilution is 1/20,000

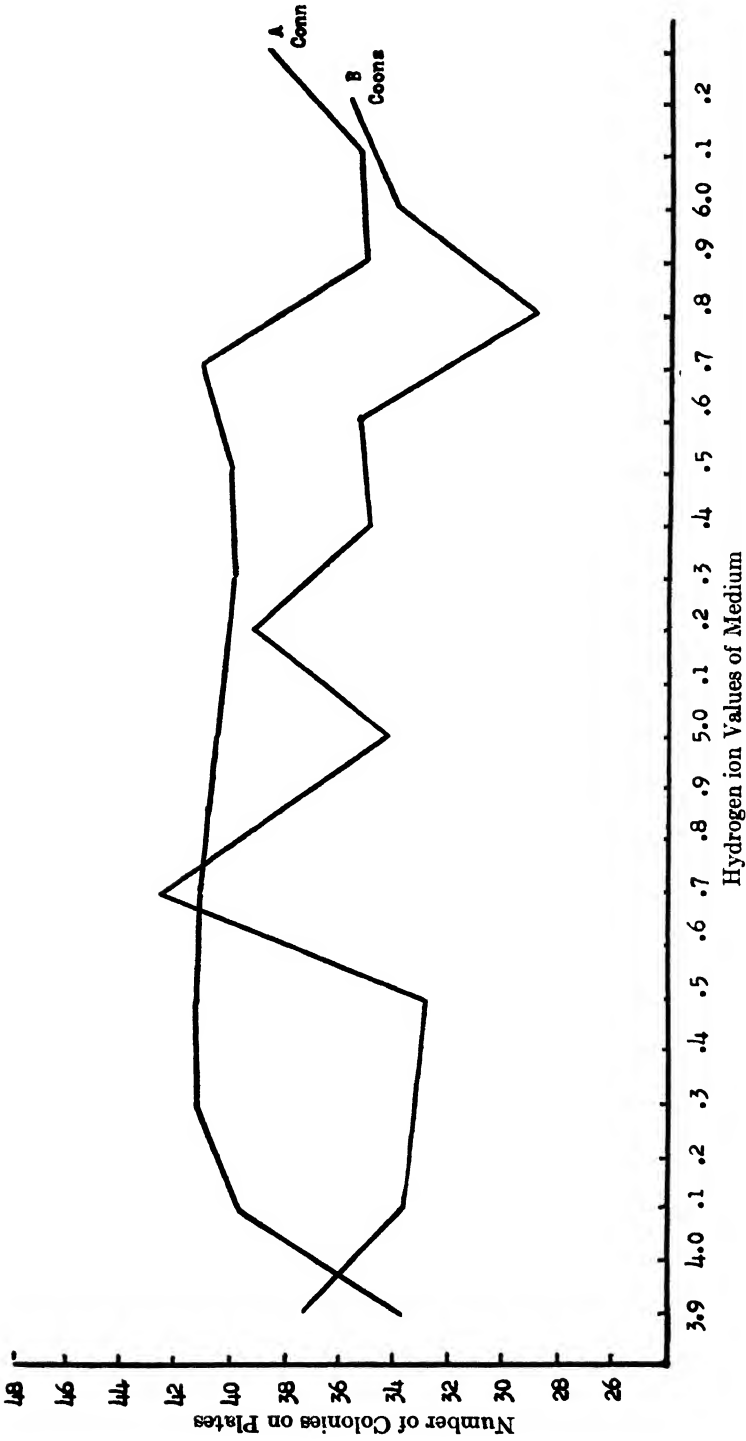


FIGURE 7.—Relation of acidity of medium to plate numbers. The results are from two different experiments. Each point is the average of plates and the dilution in both was 1/20,000

(Fig. 7-B) the determining factor of acidity being apparently overridden. In all the acidity series bacteria are practically absent at hydrogen ion values of 3.9 to 4.3. At a pH of 4.5 bacterial colonies began to appear on the plates and gradually increase in number with decreasing acidity until about a pH of 5.5 when they are fairly numerous but usually not sufficiently so to interfere with fungal development. At about a pH of 5.7 interference usually begins and in the great majority of cases lower acidity values give plates that are increasingly vitiated by bacteria so that replication of series becomes impossible. In general practice media of a pH of 4.5 to 5.7 have given the best results with the maximal numbers towards the lower acidity. With the lowering of acidity, the plates become increasingly unsightly owing to spreading bacteria and become more difficult to count. Until considerable experience has been gained there is the further disadvantage that colonies of bacteria and actinomycetes may be counted as fungal growths. The acidity of a medium changes slightly with sterilization and in all critical work on acidity values the final determinations are made or tested after sterilization whilst the medium is at a temperature of 45° C. immediately prior to pouring. In the ranges used a decrease of approximately 0.1 pH occurs on resterilization and a further reduction of about 0.1 pH on cooling.

VII. *Competition on Plates:* In addition to factors of competition already noted, there is the further complication of biological antagonism in mixed populations on plates. As there is no method of "unmixing" a mass plating this factor cannot be avoided but its importance can be greatly reduced by adoption of media which retard active growth and spreading development. Biological antagonism operates the more actively the closer the colonies are together and the more vigorously they are growing particularly in a vegetative state. Known suspensions of pure cultures plated in combination on rich media such as Beerwort or Waksman's medium, which encourage vegetative growth, show this phenomenon to a much more intense degree than equivalent suspensions on "starvation" media such as Conn's or Coon's medium. In many cases actual disappearance of small colonies already noted may ensue from the activity of some contiguous or overlying growth of different kind in a mass plating, and inhibition of germination is of common occurrence.

## F. INCUBATION FACTORS

I. *Period:* Plates maintained at 20-25° C. begin to show colonies visible to the naked eye after about 36 to 48 hours and by the third day a considerable number have appeared, the development being, to a certain extent, dependent upon the kind of medium used. The numbers gradually increase and by about the fifth to sixth day practically all the spores capable of rapid germination have produced colonies of visible size.

On "rich" media such as Beerwort, Waksman's medium or Richard's medium etc. species of *Mucor*, *Penicillium*, *Aspergillus*, *Fusarium*, *Trichoderma*, *Verticillium* and so forth rapidly overrun the plate forming large and characteristic growths which begin to fruit on the third and fourth day and by the fourth and fifth day have given rise to secondary colonies which choke the plates and vitiate further work. On "starvation" media such as those of Conn, Coon, Thornton etc. the colonies, even on the fifth and sixth day, are small and discrete and usually only just commencing to reproduce. Secondary colonies begin to appear on about the seventh to ninth day by which time there is no further germination of original fungi on the plate. The following numbers show the rate of increase of primary fungal colonies on "rich" media and on "starvation" media of equal hydrogen ion concentration the same dilution being plated throughout. The numbers stop where further counting becomes impossible.

TABLE 13.—Relation of type of medium to colony development on plates  
(Dilution = 1/20,000)

Medium	Numbers of initial colonies on successive days of incubation at 25° C.								
	2	3	4	5	6	7	8	9	10
Conn	16 <sup>a</sup>	26	41	45	49	53	55	56	
Coon	11	21	28	34	36	39	40	40	
Waksman	20	26	30	31					
Beerwort	22	28	33	35	36				

<sup>a</sup> Each number is the average of 8 plates.

By the time a small plate has developed some thirty or more colonies, the biological antagonisms set up and the staling products diffused in the medium begin to exert an intense inhibiting effect upon the development of the more slowly germinating spores and fungal moieties. Greater isolation of colonies tends to reduce this effect and in consequence plates of 23 cm. in diameter continue to produce original colonies for several days after development has ceased on a 9 cm. diameter plate of the same suspension. In most of the experiments referred to in this work the plates maintained at 25° C. were counted on the fourth, fifth, sixth and seventh days or on the third, fifth and seventh days.

II. *Temperature*: The temperature at which the plates are incubated has a marked effect upon the number of colonies that develop. This is shown in Table 14 which gives the results of duplicate series (A and B) each of eight plates receiving the same inoculum and each duplicate set maintained at different temperatures.

TABLE 14.—*Relation of temperature to number of colonies of plates**(Dilution = 1/20,000)*

Days of incubation	Duplicates	Temperatures of incubation					
		4-7° C.	13-15° C	19° C.	25° C.	30° C.	35° C.
3	A	0	8	24	26	8	5
	B	0	6	27	23	5	5
5	A	0	27	36	35	18	6
	B	1	25	34	30	13	8
7	A	1	36	42	43	25	8
	B	2	32	44	38	20	11

Usually, even after several weeks, plates at 4 to 7° C. are still producing occasional new primary colonies, the development merely being retarded by the cold, but the final numbers never approximate those in plates kept at 13 to 30° C. Colonies also develop on plates maintained at 40° C. but they are few in number. Infrequently plates at 50 and 60° C. give rise to colonies of thermophillic types of soil fungi. For all general purposes 24 to 25° C. has been adopted as the incubation temperature this giving a desirable balance of maximal numbers, rapid appearance, characterization and visibility of colonies.

## G. COUNTING FACTORS

I. *Method:* In counting the colonies which appear on the plates a sharp distinction must be drawn between fungi, actinomycetes and bacteria and only experience enables one to do this rapidly and accurately. Until this capacity is acquired, and even then, all doubtful colonies must be confirmed by examination with lens or microscope. The plates are inverted and examined through the bottom and every colony, as noted, is dotted with Indian ink on the glass in the center of the growth using a hard fine pointed pen-nib. The procedure adopted has been threefold, an initial examination under a strong opal electric bulb against a white background a further examination against a black background and a final scrutiny with the plate held obliquely in various directions against the light. Oblique illumination often shows up colonies otherwise invisible to the naked eye. The parallel nature of the curves of results of successive day's countings is an indication of the accuracy of the numbers. Where plates contain numerous colonies it is advisable to quadrate the glass bottom in fine Indian ink. In experiments where comparison of soil samples is involved all numbers should be calculated on a basis of one gram of dry soil.

## GENERAL OBSERVATIONS

As any particular experiment usually involves the comparison of several series, each of eight or more plates, it is essential that no error or personal bias which increases in one direction or is rhythmic in character, should enter into the making of the experiment. Many early experiments in the present research were vitiated by a lack of recognition of the seriousness of this factor and, perhaps still more, by a lack of recognition of the innumerable ways by which it could enter into the experimental technique. There is an inevitable tendency which often becomes consciously elaborated as a specific technique, to carry out an experiment so that each of the several manipulations are performed *seriatim* and the sets of plates are completed in order, duly labelled and put away. In the counting the method is maintained, the sets being examined in order of recording, in numerical order or in turn of increasing or decreasing values—as in an acidity or temperature series. Such tidiness and precision of methods simplifies the technique, is economical of time, prevents confusion and misplacement, facilitates recording and is a source of no little gratification to the worker. At the same time, it most effectively conceals any trend or bias of personal or experimental error, and may completely vitiate the experiment, or lead to results of unrecognized falsity. In all taking of samples, preparing of comparative suspensions or dilutions, in all pipetting, inoculation and pouring of plates and in the final counting of the colonies, the work should be carried out, not from series to series, but across the series or other experimental manipulations, randomizing as much as possible.

Further, increase or decrease of visual acuity, manipulative accuracy and so forth, depending upon physical or mental condition, freshness or fatigue etc., may have completely vitiating consequences where large series of plates have to be prepared or counted, to obtain comparative results. This factor is peculiarly important where one is counting colonies, often at the limits of vision, and where distinction has to be made between fungi, actinomycetes and bacteria. The psychological factor is one to which not nearly sufficient attention has been paid in quantitative microbiological research.

A second source of error, that underlies many of the discrepant results that have been obtained in much past work on soil mycology, has been the absence of any standardization of technique. The purpose of this paper is to emphasize the fact that variance in any one of the many factors involved in the quantitative method will give rise to variance in the final estimations of fungi per gram of soil sample. It is imperative that every single factor be standardized if results which can be duplicated and have value are to be obtained. If an impeccable and standardized technique be adopted replicated experiments give numbers of a most satisfactory degree of uniformity.

# LES CHAMPIGNONS DE MYCORHIZES ET LEUR RÔLE DANS LE DÉVELOPPEMENT DES PLANTES

J. MAGROU

*Institut Pasteur, Paris*

## INTRODUCTION

Parmi les champignons du sol, il en est qui, au lieu de vivre en saprophytes aux dépens de la matière organique morte, attaquent les organes souterrains des plantes vivantes et se nourrissent de leur substance. Certains d'entre eux altèrent et détruisent plus ou moins rapidement les tissus qu'ils envahissent; ce sont les parasites proprement dits. Mais d'autres contractent avec leurs hôtes une union intime et durable et ne paraissent pas leur nuire; en pareil cas, le mycélium du champignon et les tissus des organes souterrains de l'hôte (racines ou, plus rarement, rhizomes) sont à tel point intriqués qu'ils paraissent former un tout. On donne le nom de mycorhizes à ces organes mixtes, constitués par l'association étroite d'une racine et d'un champignon (du grec *μυκη*, champignon; *ρίζα*, racine). Chez certaines cryptogames vasculaires (Lycopodiacées, Psilotacées, Ophioglossées), et chez diverses Hépatiques à thalle, le gamétophyte est envahi de même, normalement, par des champignons filamenteux; il en résulte la formation de tissus ayant la même structure que ceux des mycorhizes, et auxquels il est d'usage d'appliquer le même nom.

Les associations que les champignons de mycorhizes contractent avec leurs hôtes rentrent dans le cadre général des phénomènes de symbiose, dont il existe d'autres exemples dans les deux Règnes. Le terme de symbiose implique, pour beaucoup d'esprits, la croyance à une association mutualiste assurant des bénéfices réciproques aux deux organismes conjoints, et se distinguant par là du parasitisme. Nous croyons préférable de rejeter ce point de vue téléologique et de prendre le terme de symbiose dans l'acception de "vie en commun d'organismes dissemblables," qui lui avait été primitivement attribuée par de Bary. Cette définition, d'ailleurs conforme à l'étymologie (*συν*, avec; *βίος*, vie), a l'avantage de ne rien préjuger de la nature des rapports unissant les organismes associés.

Si les microorganismes saprophytes du sol, par les transformations chimiques qu'ils font subir aux matériaux d'où les plantes supérieures tirent une grande part de leur nourriture, ont sur la vie de ces dernières une influence fondamentale, à plus forte raison doit-il en être ainsi des champignons qui, non contents de végéter dans le sol côté à côté avec les

autres plantes, envahissent leurs tissus et vivent en union étroite avec leurs cellules. Aussi l'attention a-t-elle été attirée de bonne heure sur le rôle que les champignons de mycorhizes peuvent jouer aussi bien dans la nutrition que dans l'évolution des plantes qui les hébergent. Nous nous proposons, après une brève description de la structure des mycorhizes, d'envisager ici ce double problème.

### MYCORHIZES ECTOTROPHES

Il existe plusieurs degrés dans l'intimité des associations que les champignons de mycorhizes contractent avec leurs hôtes. Tantôt le mycélium forme un feutrage qui entoure la racine, et ne pénètre que superficiellement les tissus de l'hôte; la mycorhize est en pareil cas dite ectotrophe. A ce type s'oppose celui des mycorhizes endotrophes, où le champignon envahit largement l'écorce de la racine et s'adapte à la vie intracellulaire.

Les mycorhizes ectotrophes s'observent communément chez les plantes arborescentes humicoles, plus rarement chez des plantes herbacées vivaces<sup>1</sup>. Leur structure a été décrite en détail par Frank (24) et, plus récemment, par M. Mangin (34). Les radicelles des arbres forestiers transformées en mycorhizes ectotrophes prennent un aspect coralloïde particulier. Elles sont entourées d'un manchon mycélien dont les filaments enchevêtrés forment un pseudo-parenchyme assez compact, d'où se détachent vers l'extérieur, des hyphes qui se dispersent dans l'humus. A la partie profonde du manchon les filaments s'insinuent entre les débris persistant de la coiffe et les cellules de l'assise pilifère, dépourvues de poils absorbants. Ils s'appliquent contre la paroi externe de ces cellules, puis s'aplatissent et s'insinuent, sous forme de palmettes digitées entre les parois latérales des cellules corticales superficielles, en dissolvant le ciment de pectate de chaux qui les unit. Chez certaines arbres, comme le chêne, le charme, le châtaignier ces lames intercellulaires ne s'étendent pas au-delà des cellules de l'assise pilifère. Ailleurs, comme chez le hêtre ou le noisetier, elles intéressent les deux premières assises de l'écorce. Chez diverses conifères (pin, épicéa, mélèze) il existe un plus grand nombre d'assises corticales à revêtement mycélien. Mais, quelle que soit son extension, le mycélium reste toujours strictement extracellulaire; jamais les lames en éventail qui s'insinuent entre les cellules n'envoient de ramifications à l'intérieur de ces dernières.

### MYCORHIZES ENDOTROPHES

Il en est tout autrement dans les mycorhizes endotrophes. On n'y retrouve plus de manchon mycélien entourant les racines: le mycélium n'aborde les radicelles qu'en de rares points, et il les pénètre aussitôt. Après avoir traversé les cellules corticales superficielles, il s'installe dans

<sup>1</sup> Par exemple chez le *Dryas octopetala* (Rosacées) (19).



la partie moyenne de l'écorce, occupant une ou plusieurs assises cellulaires; jamais il ne pénètre dans le cylindre central. Chez beaucoup de plantes, les filaments principaux du champignon restent extracellulaires, et cheminent en droite ligne dans les méats; ils s'y ramifient à angle droit, donnant de branches latérales qui pénètrent dans les cellules adjacentes et s'y résolvent en ramifications d'une extrême ténuité, constituant, par leur enchevêtrement, des buissons touffus connus sous le nom d'arbuscules. Chez d'autres espèces, non moins nombreuses, les troncs mycéliens pénètrent d'emblée dans les cellules et s'y pelotonnent; après quoi ils développent des ramifications latérales qui se résolvent en arbuscules. À un stade ultérieur, les arbuscules s'altèrent et se transforment en corps de dégénérescence que M. Janse avait décrit sous le nom impropre de sporangioles (26). Enfin, dans bien des cas, le mycélium se renfle en vésicules multinuclées, capables, comme Noël Bernard l'a observé (10), de germer en un tube mycélien, et jouant par conséquent un rôle dans la multiplication et la propagation de l'endophyte.

Chez un petit nombre d'espèces ou de familles, la structure des mycorhizes endotrophes s'écarte du type général qui vient d'être décrit. Chez les Orchidées, le champignon, toujours intracellulaire, se localise, comme à l'ordinaire, dans les assises moyennes de l'écorce, il y forme de pelotons très serrés, qui ne produisent jamais d'arbuscules ni de vésicules; certains de ces pelotons se transforment en corps de dégénérescence homologues des sporangioles.<sup>1</sup> Chez les Ericacées et les Pyrolacées, le champignon est intracellulaire, mais reste limité à l'assise la plus externe de la radicelle; il s'y ramifie abondamment, formant des buissons que M<sup>me</sup> Rayner compare à de minuscules balais de sorcière (43).

## NATURE DES CHAMPIGNONS DE MYCORHIZES

La nature spécifique des champignons de mycorhizes reste, dans bien des cas, inconnue, en raison des difficultés que présente leur culture. On avait depuis longtemps remarqué l'existence d'une relation entre les mycorhizes ectotrophes des arbres et les fructifications de divers Hyménomycètes, qui forment autour des troncs, des cercles concentriques (ronds de sorcière) ou rayonnent autour de la base de l'arbre et dessinent à la surface du sol les racines sous-jacentes (17). Grâce à des examens microscopiques approfondis, M. Peyronel (40) a constaté qu'une continuité s'établit, par l'intermédiaire de cordons mycéliens, entre les mycorhizes d'un grand nombre d'arbres forestiers et les fructifications de divers Hyménomycètes (Bolets en particulier). D'autre part, M. Melin, ayant

<sup>1</sup> Les endophytes du *Tamus communis* et du *Psilotum triquetrum* se rapprochent de ceux des Orchidées par leurs pelotons serrés et leurs corps de dégénérescence massif, mais développent en outre des vésicules et (dans le cas du *Tamus*) des arbuscules, qui établissent une transition entre le type habituel des mycorhizes endotrophes et le type aberrant représenté par les Orchidées.

inoculé à des germinations de pin du mycélium de *Boletus luteus* et de *Boletus granulatus*, a obtenu la formation de mycorhizes semblables à celles qui existent dans la nature. Cette expérience, qui réalise la synthèse du complexe mycorhizien, démontre d'une manière irréfutable que les champignons symbiotiques du pin sont bien des Bolets.

Parmi les champignons des mycorhizes endotrophes, seuls ceux des Orchidées et des Ericacées ont pu être identifiés. Noël Bernard (16) a réussi à extraire sous le microscope les pelotons intracellulaires qui infestent les racines et les embryons d'Orchidées, et à les transporter aseptiquement sur des milieux de culture appropriés. Dans ces conditions, les pelotons se développent et donnent d'emblée une culture pure d'un mycélium qui, transporté dans un semis aseptique de graines d'Orchidées, pénètre les cellules du pôle postérieur de l'embryon et y forme les pelotons caractéristiques de la symbiose. Les embryons ainsi envahis se développent et produisent ultérieurement des racines qui, au contact du champignon, s'infestent à leur tour. La preuve est ainsi faite de l'identité du mycélium obtenu en culture et de l'endophyte. Les champignons symbiotiques des Orchidées végètent en culture sous forme d'hyphes régulièrement cloisonnées, produisant tardivement des filaments moniliformes formés d'articles courts et renflés, riches en glycogène. Ces caractères permettent de les rapporter en genre *Rhizoctonia*. Noël Bernard en a décrit trois espèces; l'une (*Rhizoctonia repens*), très répandue, est commune à un grand nombre d'Orchidées; les deux autres (*R. mucoroides*, *R. lanuginosa*), caractérisées par l'existence de sclérotés développés aux dépens des filaments moniliformes sont spéciales, la première aux *Phalaenopsis* et aux *Vanda*, la seconde aux *Odontoglossum* (8). MM. Costantin et Dufour (18) ont décrit, sous le nom de *Rhizoctonia goodyerae repentis*, une quatrième espèce d'endophyte d'Orchidées, qu'ils ont isolée des racines du *Goodyera repens*.<sup>1</sup>

Les formes fructifères des endophytes d'Orchidées sont inconnues, mais un champignon très voisin (*Rhizoctonia solani*), parasite des tubercules de pomme de terre, et identique, selon Saccardo, au *Rhizoctonia violacea*, parasite des luzernes et des safrans, représente, comme l'a montré Rolfs, la forme imparfaite d'un *Hypochnus* (*H. solani* = *Corticium vagum*, var. *solani*). De même le *Rhizoctonia centrifuga*, qui forme sur les écorces d'arbres des voiles aranéeux parsemés de sclérotés, produit des formes fructifères de type *Hypochnus*. Noël Bernard a été conduit par là à rattacher les endophytes d'Orchidées à ce genre inférieur d'Hyménomycètes (8).

<sup>1</sup> M. Burgeff (12, 13) qui a confirmé les résultats de Noël Bernard, décrit sous le nom générique de *Orcheomyces* les endophytes des Orchidées. Selon lui chaque espèce d'Orchidée hébergerait une espèce distincte de champignon. D'après M. Simonet (45), les espèces fungiques décrites par M. Burgeff seraient des espèces jordanienues, entrant dans le cadre des espèces linnéennes de Noël Bernard.

Le champignon symbiotique des Ericacées a été identifié par M<sup>me</sup> Rayner (42). Chez le *Calluna vulgaris*, le champignon, contrairement à la règle générale, ne reste pas strictement localisé dans les racines; il envahit les tiges et pénètre jusque dans les carpelles, si bien qu'on le retrouve à l'état de pureté dans la cavité des fruits mûrs et dans le tégument des graines. De là, M<sup>me</sup> Rayner a réussi à le transporter aseptiquement sur des milieux nutritifs où elle obtenu son développement en culture pure. Le champignon ainsi isolé est un *Phoma* qui, inoculé à des semis aseptiques de graines de *Calluna vulgaris*, reproduit l'infestation caractéristique.

Quant aux champignons qui forment les mycorhizes endotrophes du type habituel, avec arbuscules et vésicules, il n'ont pu, jusqu'ici, être cultivés, en dépit de tentatives nombreuses. L'extraction du champignon est, en effet, dans la plupart des cas, plus difficile encore que chez les Orchidées, en raison de l'enchevêtrement des arbuscules et de la ténuité des racines infestées. Si malgré tout l'on arrive à découper aseptiquement des fragments de tissu envahi et à les semer sur des milieux nutritifs variés, on constate que le mycélium qu'ils renferment ne se développe pas; les arbuscules représentent donc, selon toute apparence, des formes beaucoup plus étroitement adaptées à la vie parasitaire que les pelotons des Orchidées. Si, d'autre part on tente de bouturer le mycélium extérieur, pris au moment de sa pénétration dans les racines, les cultures sont rapidement envahies par la flore saprophyte superficielle. Ces endophytes, par leur mycélium irrégulièrement calibré et dépourvu de cloisons, par leurs arbuscules, qui sont des sortes de suçoirs très différenciés, rappellent certains Phycomycètes parasites, et Noël Bernard (10) était tenté de les rapprocher des Peronosporées. M. Peyronel (41) qualifie de "phycomycétoïdes" les endophytes à arbuscules; toutefois, ayant observé dans une vésicule la formation de spores endogènes<sup>1</sup>, il les rattacherait plutôt aux Hémiascinées du genre *Endogone*. Mais il ne s'agit là que d'une hypothèse dont seule la culture des endophytes pourra confirmer ou infirmer la valeur.

## PHYSIOLOGIE DES MYCORHIZES

Les recherches sur la physiologie des mycorhizes ont été longtemps dominées par les conceptions de Frank (24). Frank supposait que le manchon mycélien des mycorhizes ectotrophes suppléait à l'absence des poils absorbants et favorisait la nutrition de la plante; des expériences comparatives lui montraient, à l'appui de cette manière de voir, que des plantes habituellement soumises à la symbiose prospéraient moins bien lorsqu'on les privait de leur champignon commensal. Partant de cette théorie, de nombreux chercheurs ont tenté de préciser le mécanisme de

<sup>1</sup> J'ai en l'occasion d'observer une fructification de même type chez l'endophyte d'une Hépatique (*Pellia epiphylla*) (32).

l'action favorisante du champignon, et se sont demandé notamment s'il ne jouirait pas un rôle dans la nutrition azotée de la plante, à la manière des bactéries symbiotiques des Légumineuses. Mais les nombreux travaux entrepris dans cette direction n'ont donné que peu de résultats. Si bien que les auteurs, tels que M. Gallaud (25), M. Ceillier (15), à qui l'on doit des études critiques approfondies sur le sujet, aboutissent à cette conclusion que les champignons de mycorhizes, tout au moins chez les plantes adultes, ne sont que des parasites indifférents, sinon nuisibles, en tout cas sans utilité pour les végétaux qui les hébergent.

Toutefois, la question de la fixation de l'azote atmosphérique par les champignons symbiotiques a été reprise à propos des mycorhizes des Ericacées. M<sup>lle</sup> Ternetz (46) a montré que des *Phoma* extraits des racines de diverses plantes de cette famille peuvent se développer dans des milieux de culture dépourvus de composés azotés et, dans ces conditions, fixer l'azote atmosphérique en proportion notable. Plus tard, M<sup>me</sup> Rayner (42, 43) a prouvé que les champignons étudiés par M<sup>lle</sup> Ternetz étaient bien les endophytes des Ericacées, et que des plantules asymbiotiques de *Calluna vulgaris* dépérissent dans des milieux dépourvus d'azote combine alors qu'elles prospèrent dans les mêmes milieux en présence du champignon. Ces expériences paraissent bien démontrer que les *Phoma* symbiotiques interviennent dans la nutrition azotée des Ericacées.

### RÔLE DE LA SYMBIOSE DANS LA GERMINATION DES GRAINES D'ORCHIDÉES

Une manière de voir nouvelle a été introduite dans l'étude de la symbiose par Noël Bernard. Au lieu de s'attarder à la recherche des avantages problematiques que les champignons peuvent procurer à leurs hôtes, il s'est demandé s'ils n'intervenaient pas pour modifier le développement des plantes qu'ils infestent. Ses recherches ont porté essentiellement sur les Orchidées, plantes chez lesquelles l'infestation des racines par des endophytes est de règle. Parmi les caractères aberrants si remarquables que présentent les espèces de cette famille, certains ne seraient-ils pas la conséquence de cette circonstance particulière? C'elle est la question que s'est d'abord posée Noël Bernard.

On sait que les graines d'Orchidées, réduites à un petit néanif de cellules indifférenciées, et dépourvues de réserves nutritives, sont, en règle générale, incapables de germer. Semées sur des milieux nutritifs, et dans les conditions d'humidité et de température qui suffisent à la germination de la plupart des graines, elles restent, le plus souvent, indéfiniment inertes, ou tout au plus arrivent à verdier et à gonfler légèrement.

Il arrive pourtant, exceptionnellement, dans la nature ou dans les serres, que des graines d'Orchidées germent. Ayant examiné les plantules provenant de ces germinations, Noël Bernard (3) constata qu'elles étaient constamment envahies par des champignons formant des pelotons my-

céliens intracellulaires pareils à ceux qui existent dans les racines des Orchidées adultes. Il en déduisit que les embryons ne pouvaient se développer qu'à la condition d'être pénétrés par l'endophyte. Ayant réussi à isoler ce dernier il l'introduisit dans des semis aseptiques de graines, qui ne tardèrent pas à germer en grand nombre<sup>1</sup> (5, 6). La germination succède à la pénétration du mycélium dans le pôle postérieur de l'embryon et à la formation des pelotons intracellulaires; au bout de quelques mois, on obtient dans les tubes de culture de petites plantes feuillées bien développées.

Ces premières expériences de Noël Bernard ont porté sur les *Cypripedium* et les *Cattleya*; plus tard, il a obtenu la germination, toujours au moyen des *Rhizoctonia repens*, d'un grand nombre d'Orchidées épiphytes et terrestres. Il a même réussi à faire germer les graines d'Orchidées dont la reproduction par semis était tenue pour impossible (*Odontoglossum*, *Phalaenopsis* et *Vanda*), en les inoculant avec les endophytes particuliers à ces espèces (*Rhizoctonia lanuginosa* pour les *Odontoglossum*, *R. mucoroides* pour les *Phalaenopsis* et les *Vanda*) (7, 8).

## VARIATIONS D'ACTIVITÉ DES CHAMPIGNONS

L'aptitude des champignons à faire germer les graines, leur activité en d'autres termes, est d'ailleurs variable. Elle s'atténue progressivement, et finit à la longue par disparaître tout-à-fait chez les champignons que l'on maintient en culture pure hors des Orchidées qui les hébergent normalement. Noël Bernard eut l'idée de rapprocher ce phénomène de l'atténuation de la virulence qui se produit chez les bactéries pathogènes au bout d'un certain temps de culture *in vitro*. Si le rapprochement était légitime, on devait pouvoir rendre aux champignons atténués leur activité primitive, en les accoutumant progressivement à la symbiose, de même que l'on "remonte" la virulence des bactéries atténuées au moyen de passages successifs chez des animaux sensibles. L'expérience montra qu'il en était bien ainsi (8). Un champignon devenu inactif, introduit dans un tube où l'on a semé des graines d'Orchidées, ne fait pas germer ces graines, mais est encore capable de pénétrer les embryons et d'y subsister un certain temps à l'état de pelotons. Si l'on retire ces pelotons des embryons où ils se sont formés, et si on les emploie à contaminer un nouveau semis, on constate que le champignon, après ce séjour dans les cullules de la graine, est devenu un peu plus actif; il se montre, en effet, capable de provoquer quelques germinations. Des plantules obtenues, on peut extraire de nouveaux pelotons, qui donneront un mycélium encore plus actif. En continuant ces passages en série du champignon, on arrive de

<sup>1</sup> Le milieu de culture à utiliser pour ces expériences doit fournir les aliments nécessaires aux plantules et au champignon, sans que celui-ci prenne un développement trop considérable qui étoufferait les grains. Noël Bernard s'est servi couramment de decoctions de sape de faible concentration (2 à 3 pour 1000).

proche en proche à remonter sa virulence<sup>1</sup>, et on obtient en définitive un mycélium capable de faire germer le plus grand nombre des graines d'un semis.

Il y a plus, et les semis inoculés avec un champignon inactif deviennent après quelques jours incapables de se développer quand on y introduit un champignon actif qui, à lui seul, aurait produit la germination; un champignon atténué rend donc les graines refractaires à l'action d'un champignon virulent; il se comporte à la manière d'un vaccin.

On voit par là qu'il existe un parallélisme remarquable entre la symbiose des Orchidées et de leurs endophytes, et les maladies bactériennes des animaux. Si bien que la symbiose peut être considéré comme un cas particulier de l'infection parasitaire, caractérisée par un état d'équilibre entre les actions réciproques du parasite et de l'hôte, équilibre qui rend possible le maintien indéfini de la vie commune. Il reste à voir par quel mécanisme cet équilibre s'établit et persiste de génération en génération.

### L'IMMUNITÉ DANS LA SYMBIOSE

Chez les plantules d'Orchidées, aussi bien que dans les racines des Orchidées adultes les pelotons mycéliens sont communément digérés par les cellules qui les hébergent, et transformés en corps de dégénérescence. Ce processus est à rapprocher de la phagocytose, qui, chez les animaux, détruit les microbes qui tentent de pulluler dans l'organisme. Assurément, on ne peut s'attendre à observer chez les végétaux, formés de cellules fixes et rigides, la capture des microorganismes par des éléments mobiles qui, chez les animaux, représente le premier acte de la phagocytose. Mais l'essentiel du phénomène, qui est la digestion des parasites par les cellules où ils ont pénétré, se retrouve ici avec assez de netteté pour rendre le rapprochement légitime. Aussi M. Gallaud (25), qui a étudié la digestion intracellulaire chez un grand nombre de plantes à mycorhizes, la compare-t-il avec raison à une "phagocytose sur place." Noël Bernard a précisé le rapprochement en montrant que les cellules spécialisées dans cette fonction digestive sont pourvues de noyaux multilobés, rappelant les noyaux des leucocytes dits "polynucléaires," auxquels sont dévolues, chez les animaux, les fonctions phagocytaires les plus actives (8).

Lorsque le mycélium qui envahit la graine est d'activité atténuée, la réaction phagocytaire suffit à arrêter complètement sa progression; en pareil cas, tous les pelotons sont détruits au fur et à mesure de leur formation, et la plantule s'affranchit de son parasite. Mais, vaccinée contre les atteintes des champignons actifs, elle est dès lors incapable de tout développement ultérieur. Si, au contraire, l'embryon est primitivement envahi par un mycélium actif, la phagocytose est impuissante à enrayer sa marche, et l'endophyte parvient toujours à franchir la barrière que lui opposent les phagocytes. Pourtant, dans les cas même où l'activité du

<sup>1</sup> Le terme de "virulence" est pris ici dans son sens objectif de "végétabilité *in vivo*".

champignon est suffisante pour permettre l'établissement d'une symbiose durable, une partie des tissus de la plante, et pour le moins son sommet végétatif, restent constamment indemnes d'infestation. Cette immunité persistante du sommet végétatif ne pouvant être attribuée à la phagocytose, doit tenir à l'existence, dans les sucs de la plante, d'une substance humorale capable d'arrêter la progression du parasite. Noël Bernard a proposé de voir dans le pelotonnement intracellulaire du mycélium un phénomène analogue à l'agglutination des bactéries, qui se produit communément dans les humeurs des animaux immunisés; conception d'autant plus fondée que, chez les plantules non donées d'immunité, qui se laissent envahir en totalité et détruire par le champignon, le mycélium au lieu de former des pelotons, progresse en droite ligne à travers les cellules.

La réalité de l'immunité humorale chez les Orchidées est d'ailleurs mise directement en évidence par l'expérience suivante, réalisée par Noël Bernard (9), et reproduite par d'autres expérimentateurs (31, 39): on place au fond d'un tube contenant une certaine quantité de gélose nutritive un fragment découpé aseptiquement dans le tubercule d'un *Orchis* ou d'un *Loroglossum*. Le fragment de tubercule ainsi disposé laisse diffuser dans la gélose les produits solubles qu'il renferme. Le *Rhizoctonia repens*, endophyte des Ophrydées, est semé sur la gélose à quelque distance; il commence à s'accroître comme à l'ordinaire, mais bientôt sa croissance s'arrête suivant une ligne nette et, avant qu'il ait atteint le fragment de tubercule, le mycélium dépérit à mesure que les substances diffusées se repandent dans la culture; en quelques semaines, le champignon est définitivement tué. Cette action fungicide des tubercules d'Ophrydées est spécifique; elle s'exerce bien sur le *Rhizoctonia repens*, mais non sur le *R. mucoroides*, endophyte des *Phalaenopsis* et des *Vanda*; d'autre part elle disparaît chez les tubercules chauffés à 55°. Il y a donc là un phénomène de tout point comparable à la formation des anticorps qui se développent chez les animaux immunisés et qui sont de même spécifiques et peu résistants à la chaleur.

Les réactions d'immunité ne sont pas spéciales aux Orchidées mais se retrouvent, avec les mêmes caractères, chez les autres types de plantes a mycorhizes. M<sup>me</sup> Rayner (43) a suivi les phases de la phagocytose chez le *Calluna vulgaris*; chez cette Ericacée, comme chez les Orchidées, la digestion intracellulaire paraît être en relation avec un accroissement d'activité du noyau. Le premier stade de la digestion est marqué par le pelotonnement des hyphes autour du noyau, qui augmente de volume, se déforme, devient granuleux, et se colore plus fortement par l'hématoxyline. Puis les hyphes voisins du noyau sont détruites, et la destruction se propage vers la périphérie du peloton, qui finit par se transformer en une masse amorphe et surcolorable, appendue à un tronc mycélien.

Dans les mycorhizes de type phycomycétoïde; la transformation des arbuscules en sporangioles ou corps de dégénérescence, bien étudiée par

M. Gallaud (25), suit exactement la même marche. Parfois même, le mycélium est détruit d'emblée dès sa pénétration dans la cellule, avant même que les arbuscules n'aient eu le temps de se former, et la plante se trouve affranchie de la symbiose par une réaction phagocytaire précoce et énergique. Mais il s'en faut qu'il en soit toujours ainsi; souvent, la digestion intracellulaire se limite aux arbuscules et respecte les troncs mycéliens principaux, qui, à partir de cellules où les arbuscules sont totalement dégénérés, restent capables de propager l'infestation dans les cellules voisines; la phagocytose, qui s'exerce en pareil cas de façon tardive et partielle, est impuissante à enrayer la marche du champignon, et une association stable peut s'établir (29, 30). Pourtant, dans ce cas même, le champignon reste strictement localisé dans des tissus bien déterminés des plantes; les méristèmes, le cylindre central des racines, les tubercules, les tiges aériennes et (sauf exception) les tiges souterraines restent toujours indemnes d'infestation. Il est vraisemblable que là encore interviennent des processus d'immunité humorale, qui ont pour effet de ralentir la marche des champignons en leur imposant les modes de végétation très particuliers qu'ils adoptent dans la vie symbiotique. Mais les endophytes phycomycétoïdes n'ayant pu être cultivés, il n'a pas été possible de démontrer ici, comme chez les Orchidées, la réalité de l'immunité humorale.

### LE TUBERCULE EMBRYONNAIRE DES ORCHIDÉES

On voit, parce que précède, que les rapports entre les endophytes et les plantes qui les hébergent sont de même nature que les relations qui s'établissent les microorganismes pathogènes et leurs hôtes. Si bien que la symbiose peut être définie comme la limite vers laquelle tend l'association de deux organismes antagonistes quand leurs actions réciproques s'équilibrent. Si la symbiose n'est aussi qu'un cas particulier du parasitisme, elle doit, comme toute maladie parasitaire, se manifester par des symptômes. Mais il s'agit ici d'une maladie en quelque sorte normale, commune à tous les individus ou à la plupart des individus d'une espèce donnée. Il est clair que les symptômes qui la caractérisent doivent être de même communs à tous les individus de l'espèce considérée. Ils cessent par là-même d'attirer l'attention, et se confondent avec les caractères spécifiques; c'est donc parmi ces derniers qu'ils doivent être recherchés.

Or les graines d'Orchidées, qui ne peuvent germer qu'après l'invasion de leurs cellules par le mycélium symbiotique, ont un mode de développement aberrant par rapport à celui des autres végétaux; au lieu de produire, comme les graines qui germent sans le concours de champignons, des plantules grêles, enracinées dans le sol et pourvues d'une tige à feuilles espacées, elles se renflent, dès le début de leur développement, en un petit tubercule bientôt surmonté d'un bouquet de feuilles. Noël Bernard a pensé que cette tubérisation précoce des embryons était une conséquence de la symbiose, qu'elle était en quelque sorte le symptôme de la maladie



cryptogamique à laquelle toute Orchidée est soumise dès sa germination. Il appuyait cette différenciation, les Orchidées, dépourvues de racines, n'hébergent pas de champignons; les tubercules ne se forment jamais qu'après l'apparition des racines et leur invasion par les endophytes venus du sol. Si, comme il arrive parfois (3, 23) les racines absorbantes ne se développent pas, le champignon ne peut envahir la plante, et les bourgeons, au lieu d'évoluer en tubercules, se différencient en rameaux feuillés. De cette corrélation entre la présence de l'endophyte dans les racines et la formation des tubercules, Noël Bernard avait conclu que la tubérisation des Orchidées adultes était, aussi bien que le développement du tubercule embryonnaire, une conséquence de la symbiose.

Partant de là, il n'avait pas hésité à généraliser sa théorie, en proposant de voir, dans l'apparition des tubercules, rhizomes ou autres organes pérennants des plantes vivaces, une conséquence de l'invasion des racines par un mycélium symbiotique. La tubérisation de la pomme de terre, entre autres, serait la symptôme apparent d'une modification générale du milieu intérieur de la plante par l'action de champignons endophytes vivant dans leurs organes pérennants. Cette hypothèse s'appuyait sur le fait que la symbiose, à peu près constante chez les plantes vivaces sauvages, fait généralement défaut chez les plantes annuelles. Mais cette manière de voir s'est heurtée dès l'abord à de sérieuses objections. M. E. Laurent (28) avait signalé que l'on pouvait obtenir le développement en tubercules des bourgeons d'une tige aérienne de pomme de terre coupée et plongée par sa base dans une solution de saccharose suffisamment concentrée. Les boutures ainsi traitées peuvent vivre plus d'un mois sans manière de voir sur les phénomènes de convergence que les Orchidées présentent à cet égard avec les Lycopodiacées, plantes soumises à la symbiose dès la germination de leurs spores, et dont les prothalles infestés prennent des formes renflées entoupiques semblables aux tubercules embryonnaires des Orchidées (2).

Malheureusement, le développement ne pouvant se faire dans tous ces cas qu'en présence du champignon, il paraissait impossible de vérifier cette hypothèse par des expériences de contrôle. Le cas du *Bletilla hyacinthina* a permis de tourner la difficulté. Chez cette Orchidée d'Extrême-Orient, la symbiose au début de la vie est facultative, et les graines peuvent germer avec ou sans champignons. Dans le premier cas, elles donnent des plantules élancées et grêles, analogues aux formes juvéniles de la plupart des végétaux; associées au *Rhizoctonia repens*, elles germent, comme les autres Orchidées, en un tubercule embryonnaire, dont la formation est d'autant plus précoce que l'activité du mycélium employé est plus grande.

Cette expérience montre que la tubérisation des plantules de *Bletilla hyacinthina* et, par voie de conséquence, la formation du tubercule embryonnaire des autres Orchidées, sont sous la dépendance de la symbiose. Mais, chez les Orchidées adultes, la production des tubercules continue; une

phase de différenciation, caractérisée par le développement de tiges feuillées et de racines de structure normale, alterne régulièrement avec une phase de tubérisation, caractérisée par un retard dans la différenciation histologique et morphologique des bourgeons, coïncident avec la mise en réserve des aliments non utilisés pour la différenciation. Or, au cours de la phase de développer de racines; elles absorbent directement la solution dans laquelle elles plongent par l'ouverture de leurs vaisseaux sectionnés. L'expérience réussit encore quand on assure par des procédés convenables l'aseptie de la solution et de la partie de la tige qui s'y trouve plongée.

### FACTEURS PHYSICOCIMIQUES DE LA TUBÉRISATION

Noël Bernard a repris ces expériences et en a précisé l'interprétation (4): des tiges de pommes de terre coupées sont plongées dans des solutions de glucose, de saccharose, de glycérine ou de chlorure de potassium diversement concentrées. Les bourgeons axillaires de ces boutures se développent soit en tubercules, soit en rameaux feuillés, suivant la concentration de la solution. Au-dessus d'une concentration moléculaire, ou plutôt d'une pression osmotique critique, toujours la même, quelle que soit la substance dissoute, il se forme des tubercules, tandis que, pour les concentrations plus faibles, il se développe régulièrement des rameaux. Des solutions très différentes au point de vue chimique donnent donc des résultats comparables, pourvu qu'elles soient isotoniques. Cette expérience montre que, dans ce cas, la tubérisation dépend moins de la nature chimique des substances dissoutes et de leur valeur nutritive que des propriétés physiques que les solutions tiennent de leur concentration moléculaire.

M. Molliard a montré, d'autre part, que l'on pouvait, chez diverses plantes, provoquer la tubérisation en l'absence de tout microorganisme, en élevant, par un artifice convenable, la teneur en sucres de la sève des plantes. Des graines de Radis, semées aseptiquement sur des solutions salines non glucosées, développent des racines grêles. Semées sur les mêmes solutions additionnées d'une certaine proportion de glucose ou de saccharose, elles donnent des plantules à racine principale tubérisée (36). D'autres plantes, telles que la carotte et le dahlia, se montrent incapables d'utiliser les sucres de la solution nutritive. C'est en faisant circuler dans les tubes de culture de l'air chargé de gaz carbonique, de manière à assurer une assimilation chlorophyllienne intense, que M. Molliard a pu obtenir la tubérisation aseptique de ces deux espèces (38). Chez l'oignon, dans des conditions d'éclairement convenables, la tubérisation aseptique a pu être obtenue sur des milieux très dilués (eau de source stérilisée). Les substances dissoutes nécessaires à la tubérisation sont dans ce cas fournies par la photosynthèse. Chez la pomme de terre, les plantules provenant de graines semées aseptiquement restent dépourvues de tubercules, que le milieu minéral soit ou non additionné de 5 à 10 p. 100 de glucose. Attri-

buant dans ce cas l'absence de tubérisation a une utilisation insuffisante des sucres de la solution, M. Molliard a augmenté l'absorption du glucose par les racines en suppuissant les échanges gazeux des tubes de culture avec l'air extérieur. Dans des tubes où, à cet effet, le bouchon d'ouate avait été remplacé par un bouchon de caoutchouc, les graines ont donné des plantules formées d'une tige épaisse et courte, à entre-nœuds raccourcis, à feuilles réduites à des écailles atrophiées, présentant en un mot les traits essentiels de la tubérisation; les cellules parenchymateuses de ces plantules anormales renfermaient d'ailleurs de nombreux grains d'amidon, qui font défaut dans les tiges ordinaires de la pomme de terre (37).

Tous ces exemples de tubérisation aseptique paraissent contradictoires avec la théorie parasitaire de la tubérisation. Ils montrent que la tubérisation des bourgeons d'une plante, à un moment déterminé de sa vie, dépend immédiatement de la réalisation d'un certain degré de concentration de la sève qui les nourrit en substances dissoutes. Mais, selon Noël Bernard, "la présence, dans les tissus de la plante, de parasites capables de provoquer par leurs sécrétions diastasiques le dédoublement d'édifices moléculaires complexes est une des conditions qui peuvent amener cet état. Dans les conditions naturelles de la vie cette action peut être prépondérante, et paraît l'être au moins dans certains cas" (4). En d'autres termes, la symbiose et la concentration du milieu seraient des conditions équivalentes, capables d'entraîner chez une plante donnée les mêmes effets.

### GERMINATION ASYMBIOTIQUE DES GRAINES D'ORCHIDÉES

S'il en est ainsi, on doit pouvoir substituer à la symbiose la concentration des milieux nutritifs pour obtenir la germination des graines et la tubérisation des plantules d'Orchidées. C'est ce qui arrive en effet, et Noël Bernard a réussi à faire germer des graines sans champignons, en élevant la concentration du milieu nutritif utilisé pour les semis. Ses expériences ont porté sur le *Bletilla hyacinthina* et sur les Cattlées (8). Les graines de *Bletilla* peuvent germer, comme on l'a vu, avec ou sans le concours de champignons. Semées sans champignons sur des milieux nutritifs dilués, où l'abaissement du point de congélation est égal à  $0^{\circ},01$ , elles se développent lentement en plantules grêles, à entre-nœuds allongés. Associées, sur les mêmes milieux, à des champignons d'activité croissante, elles ont un développement plus rapide et produisent un tubercule embryonnaire d'autant plus précoce et d'autant plus volumineux que le mycélium employé est plus actif. Si, d'autre part, on augmente la concentration, sans faire intervenir les champignons, on constate que, pour des concentrations encore relativement faibles (point de congélation  $\Delta = 0^{\circ},02$ ), la croissance devient plus rapide, mais se fait encore par élongation, tandis que, pour des concentrations fortes ( $\Delta = 0^{\circ},04$  et mieux

encore  $\Delta = 0^\circ, 06$ ), le mode de croissance change: la plupart des plantules présentent un tubercule embryonnaire et des entre-nœuds courts. Si l'on compare les plantules obtenues sans champignons à des concentrations de plus en plus élevées, à celles qui se sont développées à une même concentration avec des champignons de plus actifs, on constate un parallélisme étroit entre les deux séries de cultures. L'accroissement de concentration des solutions, pour les plantules élevées sans champignons, entraîne donc les mêmes résultats que l'accroissement d'activité des champignons pour les plantules soumises à la symbiose.

Les expériences sur les Cattléyées ont donné des résultats de même ordre. Semées sans champignons sur des milieux dilués, les graines de Cattléyées ne germent pas. Sur des milieux plus concentrés, elles sont au contraire capables de germination autonome et se développent en donnant des plantules tubérisées conformes au type habituel. Adaptées enfin à vivre sans champignons sur des milieux très fortement concentrés, elles développent des tubercules embryonnaires volumineux et présentant des anomalies comparables à celles que Noël Bernard a obtenues dans certains cas d'association anormales.

### CAS DE LA POMME DE TERRE

Est-il possible de retrouver, dans le cas d'une plante à tubercules telle que la pomme de terre, la même équivalence entre la symbiose et la concentration? La solution de ce problème se heurte à de grandes difficultés. D'anciennes expériences de Noël Bernard, (3) dont les résultats ont été confirmées par M. Jumelle (27), ont bien montré que l'introduction dans le sol de certains champignons commensaux habituels de la pomme de terre (*Fusarium solani*) pouvait accélérer la formation des tubercules. Mais, selon Noël Bernard lui-même, ces variations de précocité sont faibles et le phénomène reste loin de la netteté qu'on doit attendre d'une expérience pour la croire décisive. D'ailleurs, chez la pomme de terre cultivée, les mycorhizes font généralement défaut, et dans les conditions habituelles de culture, la sélection des semences et le fumure du sol paraissent suffire à assurer une production régulière de tubercules.

Par contre chez les *Solanum* vivaces sauvages tels que la douce-amère, et chez les pommes de terre sauvages (*Solanum maglia*) récoltées dans leurs stations naturelles sud-Américaines, les racines sont régulièrement envahies par un endophyte à arbuscules parfaitement caractérisé (10, 11). On pouvait dès lors se proposer d'étudier l'action de ce champignon sur le développement de la pomme de terre. Il suffit, en effet, pour obtenir la formation de mycorhizes chez la pomme de terre, d'en semer les graines au pied de douces-amères infestées. Dès que les racines des jeunes plantes ont acquis un certain développement, elles sont envahies par le mycélium. Dès lors, deux alternatives peuvent se produire: certaines plantes détruisent complètement le champignon qui tente de les envahir, et s'affranchis-

sent ainsi de la symbiose. D'autres, au contraire, se laissent largement envahir, et contractent avec l'endophyte une symbiose durable. Or le développement ultérieur de la plante diffère dans les deux cas. Les plantes affranchies de la symbiose sont, par la suite, régulièrement dépourvues de tubercules; leurs rameaux secondaires évoluent en tiges aériennes feuillées ou en longues tiges souterraines grêles. Au contraire, les plantes qui contractent la symbiose produisent toujours des tubercules développés à l'extrémité des courts stolons souterrains qui représentent les ramifications de la tige principale<sup>1</sup> (30). Assurément, en l'absence de cultures pures de l'endophyte, l'expérience ainsi conduite n'atteint pas la rigueur de celles que Noël Bernard a réalisées avec les Orchidées. Toutefois, dans tous les cas examinés, la coïncidence de la tubérisation et de la présence d'endophytes vivants d'une part, et d'autre part de la non-tubérisation et de la destruction précoce de ces mêmes endophytes s'est montrée trop constante pour qu'il puisse paraître légitime de l'attribuer au hasard. La même corrélation entre la symbiose et la production d'organes pérennants a été d'ailleurs retrouvée chez d'autres plantes à tubercules (*Orobis tuberosus*) ou à rhizomes (*Mercurialis perennis*) (30).

Chez les Ericacées, M<sup>me</sup> Rayner, ayant réussi, comme on l'a vu, à isoler le champignon symbiotique, a pu réaliser des expériences précises montrant l'influence de la symbiose sur le développement de la plante. Des graines de *Calluna vulgaris*, semées aseptiquement, après dès infection de leur tégument, sur des milieux gélosés stérilisés, germent en donnant des plantules rabougries, dépourvues de racines. Semées, toutes choses égales d'ailleurs, au contact du *Phoma* qui est l'hôte habituel de cette espèce, elles développent des plantules d'aspect normal, abondamment pourvues de racines (42).

<sup>1</sup> La relation entre la symbiose et la tubérisation a été vérifiée, dans cette expérience, par l'examen de coupes en série portant sur l'ensemble des racines chez des plantes de l'un et l'autre type. Pour chaque plante a été établi le rapport entre le nombre de radicules renfermant du mycélium vivant, et le nombre total de radicules renfermant des champignons, dégénérés ou non. Ce rapport donne en quelque sorte la mesure de l'adaptation du champignon à la vie symbiotique; il doit tendre vers l'unité dans les cas où la symbiose est réalisée, vers zéro dans le cas contraire. Voici les valeurs obtenues pour ce rapport chez les pommes de terre de semis, tubérisées ou non, étudiées par cette méthode:

Plantes tubérisées	Plantes non tubérisées
0,87	0
0,94	0
1	0,33
1	0
0,75	0
0,79	0,27
0,9	0,33
<hr/> Moyenne = 0,89	<hr/> Moyenne = 0,13

## LA THÉORIE DU PROTOCORME ET L'ORIGINE DES PLANTES VASCULAIRES

Nous avons signalé plus haut (p. 81) la convergence qui existe entre les plantules tubérisées des Orchidées et les prothalles des Lycopodiacées, soumis comme elles à la symbiose dès le début de leur développement, et comme elles renflés en toupie à l'état jeune. Chez les Lycopodiacées la tubérisation précoce peut se manifester non seulement chez le gamétophyte, mais encore chez le sporophyte. Treub (47) a attribué une importance essentielle, dans la phytogénie des plantes vasculaires, au tubercule embryonnaire ou protocorme que le sporophyte de certains lycopodes différencie précocement. Partant des résultats qu'il a obtenus chez les Orchidées, Noël Bernard a été amené à compléter et à préciser la théorie du protocorme formulée par Treub.

On sait que Treub est porté, avec d'autres botanistes, à considérer les cryptogames vasculaires d'aujourd'hui "comme les descendants de plantes ressemblant, quant à l'essentiel, aux Muscinées actuelles (plus particulièrement aux Hépatiques)" (47). Ceci admis, il se demande comment on doit se représenter les transitions entre les générations correspondantes de ces deux grands embranchements. Laissant de côté les autres aspects du problème, il cherche seulement à imaginer comment la génération asexuée des cryptogames vasculaires a pu acquérir l'autonomie physiologique qui la caractérise, "alors que, chez les ancêtres supposés de ces plantes, cette génération n'était morphologiquement autonome, vivant du reste comme parasite sur la génération sexuée."

Il est inadmissible, selon Treub, que la génération asexuée ait attendu jusqu'à la différenciation d'une racine avant de devenir physiologiquement indépendante. Longtemps auparavant, au cours de son évolution, elle aura pris des dimensions telles que la nourriture fournie par la génération sexuée ne lui suffisait plus, si bien qu'elle aura dû aller quérir elle-même dans le sol l'eau et les substances nutritives nécessaires à son développement. Ainsi, avant qu'il y ait eu des racines chez les ancêtres de nos cryptogames vasculaires actuelles, il faut que leur génération sexuée ait donné naissance à une protubérance latérale quelconque, à laquelle revenait, entre autres, le rôle de s'insinuer dans le sol et d'y puiser l'eau et les éléments nutritifs à l'aide de poils absorbants.

Ceci posé, serait-il possible de retrouver actuellement, dans l'ontogénie des cryptogames vasculaires, des traces de cet organe qui a eu une si grande importance dans leur phylogénie?

Si un tel organe, qui, dans l'évolution des plantes vasculaires aurait précédé même l'apparition de la tige feuillée et des racines, existe encore de nos jours, c'est assurément parmi les représentants les plus anciens de l'embranchement des Ptéridophytes, c'est-à-dire chez les Lycopodiacées, que l'on doit s'attendre à le remonter. Or Treub l'a précisément observé

chez le *Lycopodium cernuum*. Chez cette espèce, l'embryon différencie précocement, avant même qu'apparaissent la tige et les racines, un tubercule embryonnaire ou protocorme, qui se fixe au sol au moyen de nombreux rhizoïdes. Chez le *Phylloglossum Drummondii*, cet organe embryonnaire, qui n'a chez le *L. cernuum* qu'une existence éphémère, persiste jusqu'à l'état adulte.

Nous avons vu que chez les Orchidées, l'apparition et l'évolution du tubercule embryonnaire sont des événements dus aux progrès de la symbiose. Noël Bernard pense qu'il en est de même chez les Lycopodiacées; en effet, chez le *Lycopodium cernuum*, le protocorme, aussi bien que le prothalle, héberge un champignon que Treub a décrit et figuré. Ce serait donc par suite d'une convergence, due à la condition commune de la symbiose, qu'un protocorme serait apparu dans les deux cas. Adoptant l'idée de Treub, d'après qui le sporophyte annuel des Muscinées a acquis son autonomie en se couchant sur le sol et en s'y fixant par un protocorme, en devenant une plante vivace à tubercules, Noël Bernard la complète en admettant que l'apparition de ce protocorme, souche ancestrale du sporophyte des plantes vasculaires, est liée à l'invasion précoce de l'embryon par des champignons symbiotiques. Dans cette hypothèse, l'apparition des plantes vasculaires aurait été la conséquence d'une haute adaptation de certaines Muscinées à la vie en symbiose avec des champignons (8).

## LA SYMBIOSE CHEZ LES HÉPATIQUES

En fait, chez les hépatiques à thalle, le gamétophyte est fréquemment envahi par des endophytes, et, si la symbiose ne paraît pas dans ce cas strictement obligatoire comme chez les Lycopodiacées, elle n'en exerce pas moins une influence sur le développement de la génération sexuée. D'après M. Beauverie (1) et M. Cavers (14), le thalle du *Fegatella conica* se développe mal en l'absence du champignon symbiotique. Les spores de *Pellia epiphylla*, semées aseptiquement sur des milieux nutritifs neutres germent irrégulièrement, et les jeunes thalles auxquels elles donnent naissance ne tendent pas à périr, après un début insignifiant de développement; la culture asymbiotique de cette espèce est liée à des conditions physico-chimiques strictes; on peut, en effet, la réaliser en ajustant le milieu de culture à une réaction acide convenable ( $\text{pH} = 4.85$ ) (32, 33). Mais les thalles aseptiques de *Pellia* obtenus dans ces conditions diffèrent des thalles symbiotiques issus de spores semées sur un sol infesté; leur développement est continu et se fait sans interruption aux dépens du méristème terminal et de ses dichotomies successives. Au contraire, chez les thalles infestés, le développement est discontinu; il est interrompu par des phases de repos après lesquelles la reprise de la végétation se fait aux dépens d'ilôts méristématiques restés vivants à la face inférieure de la nervure.

Enfin, chez l'*Aneura*, M. Marcel Denis (22) a montré qu'une adaptation plus étroite que de coutume à la symbiose entraîne des modifications morphologiques des thalles, qui deviennent charnus, coralloïdes et ne renferment plus de chlorophylle. Ces thalles d'*Aneura* abondamment infestés se rapprochent des prothalles de plusieurs Lycopodes, qui sont de même charnus, coralloïdes et dépourvus de pigment chlorophyllien. On a donc là un exemple de convergence entre des gamétophytes d'hépatiques et de lycopodes, liée à une adaptation particulièrement étroite de l'hépatiques à la symbiose.

Mais si les endophytes envahissent fréquemment le thalle des hépatiques et agissent sur leur évolution, en revanche, le sporogone de ces plantes reste, en règle générale, hors de leurs atteintes. Toutefois, chez le *Pellia epiphylla*, M<sup>lle</sup> Ridler (44) a observé exceptionnellement la pénétration du champignon dans le sporophyte, dont il modifie en pareil cas le développement. Si bien que l'on peut voir, dans ces sporogones infestés de *Pellia*, l'image de ce qu'ont dû être, chez les ancêtres lointains de nos plantes vasculaires, les premières tentatives d'adaptation à la vie commune du sporophyte et des champignons, tentatives qui devaient aboutir, au cours des âges, à la symbiose obligatoire et parfaitement équilibrée dont Treub a décrit un exemple chez l'embryon du *Lycopodium cernuum*.

On ne saurait, assurément, attribuer qu'une valeur suggestive à des vues aussi largement théoriques. On doit pourtant leur reconnaître le mérite de transporter dans le domaine expérimental le problème soulevé par Treub au sujet de l'origine des plantes vasculaires. Le jour où l'on saura isoler en culture pure les endophytes des hépatiques et des Lycopodiacees, il deviendra possible d'appliquer la méthode expérimentale à l'un de ces vastes problèmes de phylogénie, qui restaient jusqu'ici confinés dans le domaine des spéculations théoriques.

### APPLICATIONS HORTICOLES ET AGRICOLES

Au point de vue pratique, quelles déductions peut-on tirer des notions sur les mycorhizes que nous venons de résumer? La découverte, par Noël Bernard, du rôle des endophytes dans la germination des Orchidées a entraîné dans l'industrie horticole une véritable révolution. On sait combien l'élevage des Orchidées était, vaguère encore, une entreprise aléatoire. Les plus recherchées de ces plantes ne pouvaient se reproduire par semés, et l'on devait, au prix des pires difficultés, aller les récolter dans les forêts tropicales où elles croissent naturellement. Pour certains genres, tels que les *Cattleya*, il était possible d'obtenir en serre la germination des graines, mais les méthodes empiriques employées à cet effet donnaient des résultats fort inconstants. On sait aujourd'hui, depuis les découvertes de Noël Bernard, qu'il suffit de disposer d'un mycélium actif pour obtenir à coup sur la germination des semences d'Orchidées les plus rebelles. Aussi les horticulteurs et les orchidophiles n'ont-ils pas hésité



à annexer à leurs serres des laboratoires, où ils procèdent à l'isolement des champignons symbiotiques, dont ils entretiennent soigneusement l'activité. Ils ont été largement récompensés de leurs efforts par les merveilleux résultats obtenus (19, 20); les Orchidées les plus rares se reproduisent aujourd'hui aisément par semis, et la méthode scientifique de culture imaginée par Noël Bernard ouvre des perspectives illimitées à la pratique de l'hybridation.<sup>1</sup>

Dans le domaine agricole, la corrélation qui a été signalée (p. 85) entre l'existence de mycorhizes et la production de tubercules et autres organes pérennants peut suggérer aussi des applications pratiques. La dégénérescence qui frappe trop souvent les cultures de pomme de terre se manifeste, entre autres symptômes, par l'affaiblissement, pouvant aller jusqu'à la suppression totale, de la récolte en tubercules. Assurément, nul ne songe à nier le rôle que jouent, dans ces maladies de dégénérescence, les virus ultra-microscopiques transmis par les Aphides. Mais les causes de la dégénérescence ne pourraient-elles être multiples, de même que les manifestations en sont diverses? Et la perte du pouvoir de tubériser ne pourrait-elle être liée au déséquilibre créé dans la plante par la disparition du champignon endophyte qu'elle héberge à l'état de nature? C'est l'idée que Noël Bernard avait formulée dès le début de ses recherches (3). M. Costantin l'a récemment reprise et développée dans une série de publications intéressantes (16). S'il en est aussi, on pourrait espérer augmenter le rendement en tubercules en ensemençant les champs de pomme de terre avec le champignon symbiotique qui est l'hôte normal de cette espèce. Malheureusement, l'impossibilité de cultiver ce champignon interdit, jusqu'ici, de recourir à cette méthode. Mais si l'on ne peut pratiquement semer le champignon au contact de la pomme de terre, ne pourrait-on, en revanche, semer la pomme de terre en des terrains où le champignon a chance de se trouver? Une enquête récente a montré que la flore mycorhizienne du sol était particulièrement abondante aux hautes altitudes (21). Partant de là M. Costantin propose, pour régénérer la pomme de terre qui, à l'état sauvage, est d'ailleurs une plante montagnarde, de la cultiver en haute montagne. Il poursuit, dans cet ordre d'idées, des essais dont les résultats paraissent concluant (16).

On voit assez que la question des mycorhizes, si elle soulève les problèmes les plus élevés de la biologie générale, mérite d'autre part, dans le domaine des applications pratiques, de retenir toute l'attention des chercheurs.

<sup>1</sup> Certains auteurs, tels que M. Bultel en France, M. Knudson aux États-Unis, tentent d'appliquer à la pratique horticole la méthode de germination asymbiotique en milieu concentré, découverte, elle aussi, par Noël Bernard (8).

## INDEX BIBLIOGRAPHIQUE

- (1) Beauverie, J. 1902. *Compt. Rend. Acad. Sci. (Paris)* 134.
- (2) Bernard, Noël. 1900. *Rev. Gén. Bot.* 12.
- (3) ———. 1902. *Ibid.* 14.
- (4) ———. 1902. *Compt. Rend. Acad. Sci. (Paris)* 135.
- (5) ———. 1903. *Ibid.* 137.
- (6) ———. 1904. *Rev. Gén. Bot.* 16.
- (7) ———. 1905. *Compt. Rend. Acad. Sci. (Paris)* 140.
- (8) ———. 1909. *Ann. Sci. Nat., Bot.* 9<sup>e</sup> série, 9.
- (9) ———. 1911. *Ibid.* 14: 223.
- (10) ———. 1911. *Ibid.* p. 235.
- (11) Bernard, (M<sup>me</sup>) N., et Magrou, J. 1911. *Ibid.* p. 252.
- (12) Burgeff. 1909. *Die Wurzelpilze der Orchideen*, Jéna, Fischer.
- (13) ———. 1911. *Die Anzucht tropischer Orchideen aus Samen*. Jéna, Fischer.
- (14) Cavers. 1904. *Ann. Bot. (London)* 18.
- (15) Ceillier, R. 1912. *Thèse Fac. des Sc. de Paris*.
- (16) Costantin, J. 1922. *Ann. Sci. Nat., Bot.*, 10<sup>e</sup> série, 4.  
1924. *Ibid.* 6.  
1926. *Ibid.* 8.
- (17) ———. 1923. *Rev. Sci. (Paris)* 8 déc., p. 733.
- (18) ———. et Dufour, L. 1920. *Rev. Gén. Bot.* 32.
- (19) ———. et Magrou, J. 1922. *Ann. Sci. Nat., Bot.*, 10<sup>e</sup> série, 4.
- (20) ———. 1922. *Nature (Paris)* No. 2539, 2 déc., p. 360.
- (21) ———. 1926. *Compt. Rend. Acad. Sci. (Paris)* 182, 26.
- (22) Denis, M. 1919. *Ibid.* 168.
- (23) Fabre, J. H. 1855. *Ann. Sci. Nat., Bot.* 4<sup>e</sup> série, 5.
- (24) Frank, B. 1885. *Ber. Deut. Bot. Gesell.* 3.  
1888. *Ibid.* 6.
- (25) Gallaud. 1905. *Rev. Gén. Bot.* 17.
- (26) Janse. 1897. *Ann. Jard. Bot. Buitenzorg.* 14.
- (27) Jumelle. 1905. *Rev. Gén. Bot.* 17.
- (28) Laurent, E. 1888. *Bul. Soc. Roy. Bot. Belg.* 26.
- (29) Magrou, J. 1920. *Compt. Rend. Acad. Sci. (Paris)* 170.
- (30) ———. 1921. *Ann. Sci. Nat., Bot.* 10<sup>e</sup> série, 3: 181.
- (31) ———. 1924. *Ibid.* 6: 265.
- (32) ———. 1925. *Ibid.* 7: 725.
- (33) ———. et Berthelot, A. 1924. *Compt. Rend. Soc. Biol. (Paris)* 91.
- (34) Mangin, L. 1910. *Nouv. Arch. du Museum d'Hist. nat.* 5<sup>e</sup> série, 2.
- (35) Melin. 1921. *Ber. Deut. Bot. Gesell.* 39.
- (36) Molliard, M. 1907. *Rev. Gén. Bot.* 19.
- (37) ———. 1915. *Compt. Rend. Acad. Sci. (Paris)* 161.
- (38) ———. 1920. *Compt. Rend. Soc. Biol. (Paris)* 83.
- (39) Nobécourt. 1923. *Compt. Rend. Acad. Sci. (Paris)* 177: 1055.
- (40) Peyronel, B. 1920. *Staz. Sper. Agr. Ital.* 53.
- (41) ———. 1923. *Bul. Soc. Mycol. France* 39.
- (42) Rayner, M. C. 1915. *Ann. Bot. (London)* 29.
- (43) ———. 1925. *Brit. Jour. Expt. Biol.* 2: 265.
- (44) Ridler, W. F. F. 1922. *Ann. Bot. (London)* 36.
- (45) Simonet. 1925. *Rev. Path. Veg. et Ent. Agr.* 12, fasc. 3, p. 204.
- (46) Ternetz, Charlotte. 1907. *Jahrb. Wiss. Bot.* 44: 353.
- (47) Treub. 1890. *Ann. Jard. Bot. Buitenzorg.* 8.

# BACTERIAL POPULATION OF SOIL

H. J. CONN

*New York State Agricultural Experiment Station, U. S. A.*

## INTRODUCTION

Considering the length of time that soil bacteria have been under investigation, there is a surprising lack of knowledge concerning the general character of the soil population. Certain groups of soil microorganisms have been given a fairly intensive study, while others have been almost completely overlooked. Those selected for the more thorough study have been chosen for one of two different reasons: Either they are organisms that grow so readily on ordinary bacteriological culture media that they produce conspicuous colonies and hence force themselves on the bacteriologist's attention; or else they are bacteria concerned in some well known transformation of plant nutrients but which can be demonstrated in soil only by some very artificial method of enrichment culture. Unfortunately, neither of these two methods of selecting cultures from the soil for study gives a true picture of the predominating flora. The following information concerning the bacterial population is, therefore, quite meager; and may well be completely modified in the future as more accurate information accumulates.

There are various methods of classifying the soil flora; but for the present purposes the most satisfactory plan seems to be to recognize two large groups on the basis of physiology, namely the heterotrophic bacteria and the autotrophic. The first of these two groups may be further subdivided into spore-forming bacteria and non-spore-forming types. This subdivision is important because the spore-formers and the non-spore-formers seem to differ not only in morphology but also in physiology. The autotrophic group might also be subdivided on this same basis; but until more is known about such organisms, it hardly seems wise to do so.

## HETEROTROPHIC BACTERIA

### SPORE-FORMING BACTERIA

The spore-forming bacteria were among the first to be recognized in soil. They include such well-known types as *Bacillus cereus*, *B. megatherium*, and *B. mycoides*, as well as many others not so easily identified. The frequency with which they have been discussed in the literature is undoubtedly due to the readiness with which they grow on culture media and the striking colonies they produce on the same. Inasmuch as their spores

are universally present in soil and are sufficiently abundant to be detected in the usual dilutions employed in making culture plates, it is almost impossible to overlook them. They were, in fact, observed in the earliest days of soil bacteriology when the methods of studying the soil flora were so crude as to overlook all forms that did not grow readily under ordinary laboratory conditions. These conditions, by the way, were originally adapted for the cultivation of pathogenic bacteria; so it is readily understandable that they did not prove especially adapted to the growth of bacteria from soil.

The first suggestion that the spore-formers were not among the active soil bacteria came from the fact that their numbers do not fluctuate when the conditions of the soil are changed. This observation led to an investigation concerning their actual method of existence in the soil, and it was found that they were practically always present as spores rather than as active vegetative forms. This information was obtained first by cultural methods, but subsequently more direct confirmation of the fact was obtained by the use of the microscope. In other words, the spore-forming bacteria are almost inactive in ordinary soil, but may be stimulated to activity by the addition of readily fermentable material especially suited to their nutritional needs. Winogradsky has recently designated such organisms as these as the "zymogenic" flora of the soil. In the case of spore-formers, the special conditions necessary to stimulate activity seem to be the presence of highly nitrogenous organic matter, together with a moderately high moisture content and moderately warm temperature.

#### NON-SPORE-FORMING BACTERIA

Non-spore-forming bacteria seem to be most abundant and presumably most important of the bacterial population. By far the greatest number of these organisms, however, are forms that have never been named and whose characteristics and functions in the soil have not yet been learned.

The best known of the non-spore-formers is *Pseudomonas fluorescens*, which seems to be a normal inhabitant of most soils, and to take part in ammonification. It seems to be particularly abundant in freshly manured soil. This organism has been recognized in the past for the same reason as the spore-formers have, namely because it grows readily in bacteriological culture media and produces large colonies on agar or gelatin that cannot be overlooked. There is reason to believe, however, that more than one actual species is covered by this term; and if the group were carefully studied, several closely related species of fluorescent liquefying pseudomonads in soil might be recognized.

Beyond this, the non-spore-forming organisms are practically unknown. Most interesting to the writer are those that are poorly adapted to growth in ordinary culture media and produce such tiny colonies on cul-

ture plates that they have often been overlooked. This group seems to be the most numerous of all the bacterial inhabitants of normal soil. They can apparently thrive almost indefinitely in moist soil without the addition of any food material and in the absence of plants. Under such conditions they may show surprisingly little fluctuation in numbers. Winogradsky calls such organisms the "autochthonous flora" and suggests that they live on the humic constituents of soil. He regards this category of organisms as being the normal inhabitants of soil, as distinct from the "zymogenic" forms which are ordinarily present in inactive state but are stimulated to very rapid multiplication when furnished with the proper conditions for growth.

The autochthonous non-spore-formers are generally small short rods, if it is possible to generalize from the comparatively small number of soils studied by the author. They are ordinarily immotile; while if motile, possess one or two polar flagella. Winogradsky, on the other hand, regards this group of organisms as being coccoid in shape. Thus there is an apparent disagreement between his findings and those of the writer. This disagreement is only apparent, however, for it proves that quite a number of these rod-shaped organisms observed in the soil studied by the writer live as rods in the laboratory for only one or two days on a fresh medium. Subsequently, they turn into coccoid form and are indistinguishable from micrococci. When inoculated into sterile soil they grow as cocci and not as rods. Hence, they undoubtedly compose a large part of the autochthonous coccoid forms observed by Winogradsky. This peculiar morphology makes them extremely interesting and more should be learned about them. Up to the present time their function has not been discovered; but there does seem to be a tendency for them to be relatively more numerous in the more highly productive soil.

### AUTOTROPHIC BACTERIA

The autotrophic bacteria are, if the term is strictly interpreted, those organisms that can live in the absence of organic matter, obtaining their carbon from such forms as carbonates or directly from atmospheric carbon dioxide. With them, however, are frequently classed those which use simple forms of organic matter like methane for their source of carbon, and those that can use elementary nitrogen, even though requiring fairly complex organic matter as a source of carbon. Such organisms as these are usually classified separately from the heterotrophic forms. This method of classification has been adopted largely for practical reasons, because these organisms must be studied by different methods from those that prefer considerable organic matter. Of recent years, however, there has been an attempt to justify such a classification on theoretical grounds. It has been assumed that the first organisms on earth must have been these autotrophic bacteria, since there was then no organic matter available as

a food supply. For this reason it is assumed they are distinctly different from the heterotrophic organisms, which have subsequently developed, and should be grouped separately. This theory is attractive, but really does not afford a complete explanation of their origin. No organic life could have preceded organic matter on the earth because even the autotrophic bacteria themselves are made up of protein. The first living organism on the earth must have obtained its original organic constituents from some source and this requires their creation by some inorganic means. Assuming therefore that organic matter was first created on earth by other than organic agency, there is no reason for insisting that the first organisms must have been autotrophic and there is no theoretical reason for classifying them separately.

It is possible, therefore, that when we understand these forms better, they will be classified with other kinds of soil bacteria. Some of the nitrogen-fixing bacteria are spore-formers and undoubtedly fall close to the other spore-forming bacteria. The non-spore-forming autotrophic organisms may, on the other hand, lie quite close to the non-spore-forming heterotrophic forms.

The exact abundance or significance of the autotrophic organisms in soil is not yet known. The nitrifiers and nitrogen-fixing organisms have always been assumed to be very important. It must be recognized, however, that they are not ordinarily abundant in soil, and that to obtain cultures of them soil must be submitted to very artificial conditions. Such considerations as this make it doubtful whether they may be of such great significance as generally assumed. Someone may some day discover that there are other organisms in soil more numerous than these autotrophic forms which are actually concerned in the processes generally ascribed to the latter. There are, in fact, so many heterotrophic organisms in soil which are very abundant, yet whose physiology is still unknown, that it seems rather improbable that the most important biochemical activities in soil are carried on by the much less abundant autotrophic bacteria, as has generally been assumed in the past.

# THE EFFECT AND IMPORTANCE OF THE ABSORBING COMPLEX (HUMUS-ZEOLITE) IN SOILS AS REGARDS SOME IMPORTANT SOIL BACTERIA

A. A. J. DE'SIGMOND, L. TELEGDY-KOVÁTS AND F. ZUCKER  
*University of Technical Science, Budapest, Hungary*

## INTRODUCTION

From consideration of the theoretical principles and of the effect of the saturation of the absorbing complex (zeolite-humus) and of the quality and quantity of the absorbed cations on the physical properties of the soil, we might have predicted in advance some of the effects of the zeolite-humus-complex on some important soil bacteria. The following paper will report very instructive experimental results which support this supposition.

In the soil laboratory of the Royal Hungarian Joseph University of Technical Science, the authors have investigated first, the effect of the artificial zeolites (permutitei) on nitrifying bacteria (*Nitrosomonas* and *Nitrobacter*) and on different *Azotobacter* cultures. The chemical composition of the permutite was changed in three essentials as follows: sodium permutite, calcium permutite and completely unsaturated permutite. The latter was produced from the calcium salt by continuous washing with water saturated with carbon dioxide until no calcium could be detected in the last portion of the filtrate.

In regard to the *Nitrosomonas* pure culture, produced according to Winogradsky, after numerous qualitative tests the quantitative experiments proved that:

## DISCUSSION OF RESULTS

(1) The calcium permutite very considerably increased the oxidation of ammonium sulfate to nitrite; as in the case when 4 g. of calcium permutite was added 62.72 per cent of the ammonium sulfate turned to nitrite whereas in the check experiment only 43.15 per cent was converted.

(2) The sodium permutite, on the contrary, had a depressing effect on the production of nitrite.

(3) The completely unsaturated permutite caused complete failure of the production of nitrite. In the case when a half part of calcium permutite was added to a half part of unsaturated permutite the production of

nitrite was the same as in the case of calcium permutite alone, showing that the failure in the preceeding experiments was due to the deficiency of calcium plant food and not to some harmful effect of the unsaturated permutite.

With the pure cultures of *Nitrobacter* similar experiments were carried on and as a whole the results have been similar. But the *Nitrobacter* cultures proved less sensitive than the *Nitrosomonas* cultures.

As regards the hydrogen ion concentration of the culture media, the pH was varied from about 7 to 10, and except around the limiting values, the harmful effect was not noticeable.

Further particulars are to be found in the inaugural dissertation of Zucker (4).

In the experiments with *Azotobacter* cultures the results are very similar to the previous ones. The sodium permutite more or less prevented the fixation of nitrogen depending upon its quantity. The calcium permutite proved, on the contrary, very beneficial. From direct comparative experiments we can state, that the calcium of the zeolite was at least as available to the *Azotobacter* cultures as that of calcium carbonate or calcium malate, whereas that of calcium chloride, calcium sulfate, monocalcium phosphate, dicalcium phosphate and calcium phosphate did not prove available. It seems that the *Azotobacter* cultures cannot use the calcium in combination with strong mineral acids, only that combined with weak organic or inorganic acid. This evidence reaffirms the acidoid, weaker than phosphoric acid.

The completely unsaturated permutite prevented any nitrogen-fixation. It seems that the chief rôle in this case was the absence of calcium food, because the pH of the culture media ran around 7.23 to 7.34, in no case so low as to be harmful to nitrogen-fixation.

We know, that a pH of 6.2 to 8.8 does not interfere with nitrogen-fixation though the optimal limits are about a pH of 7 to 8 (3). The lowest limit for growth seems to run around a pH of 5.9 to 6.0 (1), and the nitrogen-fixation commences in the above experiments only in the presence of monocalcium phosphate and continues until a pH of 5.07 is reached at which point there was no growth at all; in all the other experiments the pH was above 7, some times very much above 7, some times reaching 11.46. It was the object of these experiments to find the highest limit, but even at this high pH nitrogen-fixation, though considerably slowed down did not stop; about 2.368 to 3.975 mg. of nitrogen per gram of mannite being fixed. Nevertheless, we could notice that above a pH of 8.10 the fixation of nitrogen often dropped back, if fixation was caused by sodium permutite. On the other hand, in the presence of calcium permutite or calcium carbonate a pH of 9.50 to 9.99 did not do any harm. It seems as if the presence of available calcium makes the *Azotobacter* cultures more resistant to a high pH.



Further details are reported in the inaugural dissertation of L. Telegdy-Kováts (2).

### INVESTIGATION OF TYPICAL BACTERIA GROUPS

We have investigated the number of typical soil bacteria groups in three different soil types: (1) garden soil in good tilth and fertility; (2) a typical "Szik"—soil of the solonetz type; (3) a typical alkali soil of the Solonchack type.

Soil No. 1 was neutral with a pH of 6.8 and contained 6.9 per cent calcium carbonate. The moisture of the soil at the time of inoculation of the culture media was 22.4 per cent. In soil No. 2 the pH was 6.5, calcium carbonate was deficient and the moisture was 9.2 per cent. In soil No. 3 the pH was 9.4, total salt content (by electrolytic conductivity) was 0.5 to 1.0 per cent with 0.28 per cent sodium carbonate and rich in calcium carbonate per cent moisture.

The number of the nitrogen-fixing aerobic and anaerobic bacteria, as well as the nitrifying and denitrifying bacteria are shown in the following figures:

	Soil No. 1	Soil No. 2	Soil No. 3
Nitrogen-fixing, aerobes	10,000	10	None
Do Do anaerobes	10,000	1000	1000
Nitrifying bacteria	10,000	none	none
Denitrifying bacteria	100	1000	100

In both alkaline soils (Nos. 2 and 3) the nitrogen-fixing aerobes were few because in both cases the soil was very compact; the anaerobes did thrive but not so well as in the garden soil. It is noticeable that in the case of both alkali soils no nitrification was detected. This question was studied on numerous other alkali soils and except in the case of spots with barnyard manure no nitrification could be detected. This fact may be due especially to the compactness of these soils. It is noteworthy, that in the sandy alkali soil near Szeged and elsewhere the saltpeter efflorescence was very often found and used in earlier times for gun powder. But the permeability of these soils and the high calcium carbonate content cause the conditions which are different from those of the above compact alkali soils.

At the same time the following bacteria were detected in the above three soils:

	Soil No. 1	Soil No. 2	Soil No. 3
Ammonifying bacteria	100,000	10,000	1000
Carbon decomposing bacteria	100,000	10,000	1000
Protein decomposing Do	10,000	10,000	1000
Cellulose Do Do (aerobes)	10,000	10,000	10,000
Do Do Do (anaerobes)	1000	100	10
Butyric acid producing Do (anaerobes)	100,000	10,000	1000
Pectin decomposing Do (aerobes)	100,000	10,000	10,000

The abundance of each class of bacteria in the garden soil No. 1 is evident. As the soil is a neutral garden soil of good tilth, we can suppose that the zeolite-humus-complex is mainly saturated with the calcium cation, hence favorable for bacterial life. Soil No. 2 is a solonetz with solotisation commenced, consequently the absorbing complex is partly saturated with sodium and hydrogen ions and the quantity of the calcium cations is reduced, hence all those bacteria requiring calcium are more or less deficient. In the case of soil No. 3 the high alkalinity together with the sodium carbonate content may be responsible for the deficiency of most of the bacteria.

We are not willing to draw other conclusions, because we consider the above data only as preliminary experiments. But this method of experimenting is promising and taken with the previous exact investigations we feel entitled to suppose that the zeolite-humus-complex because of the kind and degree of saturation would have a decided influence on the bacterial flora of the soil.

#### LITERATURE CITED

- (1) Gayney and Batchelor. 1922. Influence of H-ion on growth of *Azotobacter*. *Ibid* 16: 49 and 56.
- (2) Telegdy-Kováts, L. 1927. The influence of several artificial zeolites on the nitrogen-fixation by *Azotobacter* cultures. Inaug. Dissertation Budapest, Hungary.
- (3) Waksman and Karumaker. 1924. Nitrogen-fixation and mannite decomposition. *Soil Sci.* 18: 380.
- (4) Zucker, F. 1927. The process of nitrification in presence of a few artificial zeolites. Inaug. Dissertation.

# MICROORGANISMS IN SOME SOIL PROFILES IN IOWA

P. E. BROWN AND T. H. BENTON

*Iowa Agricultural Experiment Station, U. S. A.*

## INTRODUCTION

Investigations on the numbers of bacteria in the soil at different depths have been carried out at various times in the past and some interesting and important conclusions have been drawn from these studies. The earliest work along this line, which has been reviewed by Waksman<sup>1</sup> indicated that the greatest number of organisms occurred near the surface of the soil and that the lower soil layers contained very much smaller numbers. The decrease was very rapid in some soils and more gradual in other cases and bacteria were found at much lower depths in certain instances than in others. The work of Brown<sup>2</sup> and that of Waksman, referred to above are the only studies, however, which have been made on individual soil types and in these cases counts were made at arbitrary depths, with no regard for the natural variations in soils in different layers.

The recent development of interest in soil classification and the recognition of the fact that soils must be described according to their profile characteristics, by horizons has led to a more careful study of the various soil layers from the chemical and physical standpoints. It is apparent that complete information regarding the character of the various horizons of the profile of any soil type cannot be secured until they are studied bacteriologically.

The work reported here was begun to determine the content of microorganisms in the various soil layers of some typical Iowa soils. Studies were made on Carrington loam and Lindley sandy loam, two important Iowa soils, the former being one of the most extensively developed types in the state. Samples were taken from the various horizons, with the usual precautions and the numbers of bacteria, actinomycetes and molds were counted on agar plates. The ordinary dilution method was followed and Brown's Egg Albumen agar was used for the bacteria and actinomycetes and Waksman's Synthetic acid mold medium was employed for the molds.

Samplings were made of typical profiles of Carrington loam in cultivated areas on June 20, June 25, July 23, August 25, September 20, October 21, and November 11, 1926, of a typical profile of a virgin Carrington loam on June 20 and of profiles of Lindley sandy loam on September 20, October 21, and November 11. The moisture content of the samples was

<sup>1</sup> Soil Science 1: 363.

<sup>2</sup> Research Bulletin 8, Iowa Agr. Exp. Sta.

determined in all cases and the results are calculated on an air-dry basis. The data are given in Table 1.

### DESCRIPTION OF SOIL PROFILES

Very careful descriptions were made of each profile studied and some of these are given below, only one being shown for the cultivated Carrington loam, one for the Carrington loam in sod, and one for the Lindley sandy loam. They are typical of the other samples.

#### CARRINGTON LOAM, NUMBER 1

- 0 to 2 in.—Very dark grayish-brown friable loam containing considerable fine sand. When wet soil appears very dark brown to almost black. Structure is finely granular.
- 2 to 12 in.—A very dark grayish-brown mellow loam. Color seems uniform and almost solid. Has a high per cent of silt-fine granular structure.
- 12 to 18 in.—Transition zone. Changes in color from a very dark grayish-brown to moderately dark grayish-brown. Somewhat heavier in texture and almost a silty clay loam, friable, fine, granular structure.
- 18 to 26 in.—Brown light silty clay loam, friable, discolored somewhat by organic infiltration but not as much as layer above. The structure is indefinite with a tendency to finely granular.
- 26 to 36 in.—Yellowish-brown gritty clay loam. Not as heavy as layer above, at least seems more friable, and crumbly. A few faint organic infiltrations are present. Iron stains occasional but faint. Pieces of small gravel occur infrequently, with considerable coarse and fine sand, which form only a small per cent of the total soil layer. Faint gray mottlings noticeable.
- 36 to 50 in.—Yellowish-brown clay loam, quite gritty, with gray splotches and iron stains quite abundant. Coarse drift materials more abundant in this layer. Very much like layer above in color except for an increase in gray and more iron stains.

#### CARRINGTON LOAM, NUMBER 2

- 0 to 3 in.—A grayish-brown smooth friable loam well filled with a dense interlacing of grass roots. When wet, the soil appears dark brown to almost black. The soil has a medium granular structure, the granules clinging to the grass roots.
- 3 to 14 in.—A very dark grayish-brown mellow loam, smooth and friable, indicating a considerable amount of silt and very fine sand. Grains of coarse sand are prominent with an occasional piece of small glacial gravel. Organic infiltration and coloration give this an almost black appearance when wet. When pulverized, the soil is grayish-brown in color.

TABLE 1.—*Microorganisms in some soil profiles in Iowa*

Soil type	Date sampled	Depth soil horizon in inches	Per cent H <sub>2</sub> O	Bacteria per gram air-dry soil	Actinomycetes per gram air-dry soil	Molds per gram air-dry soil
Carrington loam Number 1 (cultivated)	June 20, '26	0-2 A <sub>1</sub>	9.0	8,830,000	770,000	17,100
		2-12 A <sub>2</sub>	16.0	5,710,000	710,000	17,300
		12-18 A <sub>3</sub>	15.0	3,650,000	700,000	15,500
		18-26 B <sub>1</sub>	14.0	372,000	40,000	560
		26-36 C <sub>1</sub>	13.0	42,900	32,000	520
		36-50 C <sub>2</sub>	14.0	4,380		53
Carrington loam Number 2 (virgin sod)	June 20, '26	0-2 A <sub>1</sub>	6.0	3,636,000	425,000	11,700
		2-14 A <sub>2</sub>	8.0	4,565,000	652,000	10,300
		14-19 A <sub>3</sub>	9.0	3,510,000	582,000	9,450
		19-24 B <sub>1</sub>	10.0	730,000	44,000	2,610
		24-50 C	11.0	4,300	33	190
Carrington loam Number 3 (cultivated)	June 25, '26	0-2 A <sub>1</sub>	7.0	4,190,000	1,070,000	21,500
		2-6 A <sub>2</sub>	10.0	5,000,000	900,000	16,600
		6-16 A <sub>3</sub>	12.0	4,660,000	680,000	13,800
		16-25 B <sub>1</sub>	14.0	837,000	93,000	1,390
		25-32 C <sub>1</sub>	16.0	37,000	5,900	470
		32-50 C <sub>2</sub>	17.0	7,100	840	72
Carrington loam Number 4 (cultivated)	June 25, '26	0-2 A <sub>1</sub>	9.0	4,725,000	988,000	17,500
		2-6 A <sub>2</sub>	11.0	4,740,000	809,000	17,900
		6-14 A <sub>3</sub>	11.0	4,440,000	560,000	11,700
		14-24 B <sub>1</sub>	17.0	614,000	84,000	2,160
		24-33 C <sub>1</sub>	18.0	36,500	6,000	720
		33-50 C <sub>2</sub>	17.0	7,710	730	48
Carrington loam Number 5 (cultivated)	July 23, '26	0-2 A <sub>1</sub>	12.0	3,300,000	909,000	29,500
		2-6 A <sub>2</sub>	16.0	3,900,000	1,070,000	28,500
		6-14 A <sub>3</sub>	17.0	3,700,000	590,000	16,800
		14-20 B <sub>1</sub>	17.0	687,000	204,000	2,000
		20-34 C <sub>1</sub>	16.0	26,500	4,760	1,900
		34-50 C <sub>2</sub>	15.0	5,000	705	294
Carrington loam Number 6 (cultivated)	July 23, '26	0-2 A <sub>1</sub>	10.0	3,550,000	660,000	21,100
		2-6 A <sub>2</sub>	11.0	4,720,000	590,000	18,600
		6-12 A <sub>3</sub>	12.0	4,900,000	610,000	16,000
		12-19 B <sub>1</sub>	13.0	356,000	69,000	1,700
		19-32 C <sub>1</sub>	16.0	17,800	5,400	590
		32-50 C <sub>2</sub>	17.0	2,860	1,370	250
Carrington loam Number 7 (cultivated)	Aug. 25, '26	0-2 A <sub>1</sub>	11.0	3,146,000	560,000	26,900
		2-6 A <sub>2</sub>	15.0	2,059,000	529,000	25,800
		6-15 A <sub>3</sub>	16.0	2,500,000	476,000	18,900
		15-26 B <sub>1</sub>	18.0	414,000	134,000	2,300
		26-35 C <sub>1</sub>	19.0	24,600	3,700	860
		35-50 C <sub>2</sub>	19.0	5,300	240	220
Carrington loam Number 8	Aug. 25, '26	0-2 A <sub>1</sub>	15.0	4,101,000	700,000	23,500
		2-6 A <sub>2</sub>	17.0	4,337,000	600,000	21,600
		6-18 A <sub>3</sub>	20.0	3,625,000	560,000	17,500
		18-28 B	19.0	456,000	148,000	1,480
		28-38 C <sub>1</sub>	18.0	72,900	4,260	1,460
		38-50 C <sub>2</sub>	18.0	6,590	850	207

TABLE 1 (Continued).—*Microorganisms in some soil profiles in Iowa*

Soil type	Date sampled	Depth soil horizon in inches	Per cent H <sub>2</sub> O	Bacteria per gram air-dry soil	Actinomycetes per gram air-dry soil	Molds per gram air-dry soil
Carrington loam Number 9 (cultivated)	Sept. 20, '26	0-2 A <sub>1</sub>	10.0	4,330,000	880,000	25,500
		2-6 A <sub>2</sub>	12.0	4,880,000	790,000	22,700
		6-16 A <sub>3</sub>	15.0	4,000,000	580,000	17,600
		16-24 B	16.0	309,000	35,000	1,900
		24-35 C <sub>1</sub>	17.0	38,500	4,800	1,320
		35-50 C <sub>2</sub>	17.0	7,600	830	325
Lindley sandy loam Number 10 (cultivated)	Sept. 20, '26	0-2 A <sub>1</sub>	5.0	3,580,000	631,000	16,800
		2-6 A <sub>2</sub>	8.0	3,910,000	543,000	22,700
		6-16 A <sub>3</sub>	10.0	3,440,000	550,000	13,300
		16-24 B	10.0	466,000	22,200	2,000
		24-35 C <sub>1</sub>	16.0	33,700	4,650	1,620
		35-50 C <sub>2</sub>	15.0	9,500	580	130
Carrington loam Number 11 (cultivated)	Oct. 21, '26	0-2 A <sub>1</sub>	11.0	3,370,000	560,000	19,100
		2-6 A <sub>2</sub>	12.0	3,630,000	569,000	18,100
		6-14 A <sub>3</sub>	16.0	3,330,000	410,000	11,900
		14-21 B	14.0	510,000	116,000	1,300
		21-27 C <sub>1</sub>	16.0	57,000	5,900	470
		27-50 C <sub>2</sub>	15.0	6,230	235	153
Lindley sandy loam Number 12	Oct. 21, '26	0-2 A <sub>1</sub>	8.0	3,600,000	430,000	15,200
		2-6 A <sub>2</sub>	9.0	3,840,000	656,000	17,500
		6-14 A <sub>3</sub>	11.0	3,480,000	449,000	17,400
		14-21 B	12.0	545,000	79,500	2,100
		21-27 C <sub>1</sub>	17.0	47,000	7,200	720
		27-50 C <sub>2</sub>	15.0	7,500	350	200
Carrington loam Number 13 (cultivated)	Nov. 11, '26	0-2 A <sub>1</sub>	16.0	5,119,000	950,000	23,800
		2-6 A <sub>2</sub>	19.0	5,000,000	1,110,000	25,900
		6-15 A <sub>3</sub>	20.0	4,125,000	1,000,000	13,700
		15-18 B	17.0	237,000	151,000	1,920
		18-32 C <sub>1</sub>	13.0	59,800	10,000	2,520
		32-50 C <sub>2</sub>	12.0	2,100	1,930	204
Lindley sandy loam Number 14 (cultivated)	Nov. 11, '26	0-2 A <sub>1</sub>	7.0	5,270,000	1,180,000	12,900
		2-6 A <sub>2</sub>	9.0	4,830,000	990,000	25,200
		6-15 A <sub>3</sub>	10.0	4,060,000	660,000	40,000
		15-18 B	10.0	751,000	110,000	3,110
		18-32 C <sub>1</sub>	18.0	60,000	6,800	2,920
		32-50 C <sub>2</sub>	15.0	12,000	1,130	236

14 to 19 in.—Brown heavy loam to light silty clay loam, but so affected by organic matter that it has the appearance of a dark grayish-brown—not a solid color. Some lighter colored subsoil has been brought up through worm burrows—fine granular structure 1 to 16 inches—then becomes a looser structure.

19 to 24 in.—More uniformly brown in color than above. Texture a silty clay loam, discolored considerably by infiltration of organic matter. A few grass roots still present. Almost structureless, tends to be finely granular, very pulverent.

24 to 50 in.—Yellowish-brown silty clay loam, practically structureless but tends to be finely granular. Color almost solid. Some organic

infiltration, faint gray mottling and iron stains gradually becoming more pronounced with depth. The layer is quite gritty, containing considerable coarse sand and occasional gravel. Rotten greenstone, small black coarse sand deposits, and other drift materials, with an occasional small boulder imbedded is found. Below 50 inches the mottling becomes very pronounced with gray splotches and many orange brown iron stains.

Virgin sod—uncultivated apparently since first settlements.

#### LINDLEY SANDY LOAM, NUMBER 10

- 0 to 2 in.—Light grayish-brown sandy loam containing a high per cent of fine sand and with small gravel scattered occasionally over the surface, single grain structure.
- 2 to 6 in.—Light grayish-brown friable sandy loam—has some brown to dark brown organic coloration in occasional fine streaks and thread-like lines, but very faint.
- 6 to 16 in. or 18 in.—Light brown sandy loam containing more silt and clay than the upper horizon, no structure. Pseudo mottlings or streaks of gray and brown, very faint (not true mottlings).
- 18 to 24 in.—Heavy gritty silty clay, light brown in color with a few gray and brown mottlings. Irregular granular structure.
- 24 to 33 in. or 36 in.—Heavy plastic silty clay, light brown (a shade lighter than horizon above) containing a few gray and brown mottlings. Considerable coarse sand and fine rock particles incorporated. A few iron stains present. Irregular granular to platy.
- 36 to 50 in.—Yellowish-brown (lighter brown than overlying horizon) silty clay or clay, mottled gray and yellowish-brown. Contains much grit, coarse sand and gravel particles. Numerous iron stains. Irregular granules and plates, indefinite structure.

#### EXPERIMENTAL RESULTS

Examining the results in Table 1, it is evident that there is some variation in the content of bacteria in the surface horizon of cultivated areas of Carrington loam at the different dates of sampling. How much of this variation is due to seasonal changes and how much to normal soil variations, it is impossible to say. It is believed, however, that the seasonal effects are of major importance and the differences in various areas of typical Carrington loam are of quite minor significance. On June 20, there were over eight million bacteria in the surface two inches of the soil. The lowest count at this depth was secured on August 27. Considerable variation also occurred at the lower depths and in the lowest horizon the number of bacteria ranged from 2,110 per gram up to 7,710 on June 25. In several cases there were more organisms present in the  $A_2$  horizon than in the  $A_1$ , which is in accord with previous observations. In some in-

stances there was little difference in the number of bacteria present in the three surface horizons but in all cases, there was a very large decrease in numbers in the B horizon.

The number of actinomycetes varied more widely than the bacteria in the surface soil, ranging from 560,000 per gram on August 25 and October 21 up to 1,070,000 on June 25. In some cases there were more variations in the A horizons than was true with the bacteria and the largest counts were frequently found in the lower parts of the A horizon. As with the bacteria, however, the greatest decrease occurred in the B horizon and only a few organisms occurred in the lowest horizon, the number here varying from 235 per gram up to 1,930.

The mold content of the surface horizon varied from 17,100 up to 29,500 per gram. In several cases the A<sub>2</sub> horizon was higher than A<sub>1</sub> but in general there was little difference between these horizons. The A<sub>3</sub> horizon, however, was always much lower, and a very large decrease occurred in the B horizon. A few molds were found in all cases at the lowest depths but the number there was not very widely different in the various samples.

Comparing the results on cultivated Carrington loam and on the same type under virgin sod, as sampled on June 20, it appears that there is a much smaller number of bacteria in the A horizon, and a smaller number of actinomycetes and molds. The B horizon, however, is higher in bacteria and molds and much the same in actinomycetes. The lowest horizons are similar. The number of bacteria and actinomycetes is greater in the A<sub>2</sub> horizon of the virgin soil than in the A<sub>1</sub>, while the reverse was true in the case of the cultivated Carrington loam. Later samplings of other profiles of typical Carrington loam, however, occasionally show more organisms in the A<sub>2</sub> horizon so that this difference may not be entirely due to the cultivation factor. It would seem, however, that the cultivation of the Carrington loam plays a very important part in determining the bacterial content of the soil, not only in the surface horizon but also in the lower soil layers. Land in sod is always low in microorganisms at the surface when compared with cultivated areas of the same type.

Considering now the results for the Lindley sandy loam in comparison with the Carrington loam on three different dates, it would seem that the two soils are very similar in bacterial content although the Lindley seems to have larger numbers in the B and C horizons, with one or two exceptions, while the surface horizon of the Carrington loam was higher except on November 11. There is a considerable difference in the number of actinomycetes however, the Lindley sandy loam being generally lower than the Carrington loam. There are one or two exceptions but in general the Carrington is richer in actinomycetes at all depths. The mold content is also lower in the Lindley and in general at all depths but there are some exceptions here. In some cases the differences are too small to be significant.



## SUMMARY

It would seem from these results that the content of *microorganisms* in the various horizons of typical soil profiles may be rather closely *correlated* with the other characteristics of the horizons. The numbers *vary* in typical samples on through the season and seasonal effects on the various groups of organisms are undoubtedly of considerable significance. Minor variations occur in content of microorganisms in different profiles of the same type but greater differences occur between types, and especially at the lower horizons and in the content of actinomycetes and molds. Treatment of the soils affects the microorganic content and may influence numbers to considerable depths. Soil in sod is much lower in microorganisms than cultivated areas, differences occurring throughout the soil profile, larger numbers being found in some cases in the B horizon and wider differences in distribution occurring through the A horizon.

Further studies on the activities of microorganisms in the various soil layers are very desirable but these results indicate that the numbers of organisms may show something of the character of the individual soil horizons and incidentally point out the changes which are going on in the soil.

This work is being continued on a rather extensive scale.

# THE OCCURRENCE OF AZOTOBACTER IN SOIL<sup>1</sup>

P. L. GAINES

*Kansas Agricultural Experiment Station, U. S. A.*

## INTRODUCTION

Following the isolation of *Azotobacter chroococcum* by Beijerinck (5) the presence of this species and another closely related free-living aerobic nitrogen-fixing organism was noted by many different investigators in soils taken under a great variety of conditions in widely separated localities. No effort will be made to call attention to the numerous instances mentioned in the literature where organisms belonging to this group have been detected in soils. Suffice it to say, that the accumulated evidence seems to indicate that strains or species of *Azotobacter* are present in certain soils of virtually every country from which soils have been examined.

It is the purpose of this paper to call attention to the relative frequency with which *Azotobacter* are encountered in soils when a more or less systematic search is made for them. Also to call attention to the various explanations that have been offered to account for their absence from certain soils.

## THE OCCURRENCE OF AZOTOBACTER IN SOILS AND EXPLANATIONS FOR ITS ABSENCE

Most of the earlier records, such as cited by Omeliansky and Solonushkoff (34), mention only those instances where *Azotobacter* were noted. However, during more recent years a number of investigators have recorded not only those soils giving a positive, but also those giving a negative *Azotobacter* test.

Heinze (25) was among the first to study the distribution of *Azotobacter*. He noted the presence of these organisms in soils from a variety of conditions, including cultivated, forest, meadow, and mountain soils of southern Europe, but failed to record negative results if any were observed. His work would indicate a wide distribution including soils up to an elevation of 2100 m.

Burri (7) is quoted by Waksman (45) as having examined 105 samples of soil and found *Azotobacter* missing in 34 cases, mostly heavy clay soils.

Fischer (16) examined 17 variously treated soils and observed *Azotobacter* in all but one. He expressed the opinion that the presence of

<sup>1</sup> Contribution No. 97, Department of Bacteriology.

calcium oxide to the extent of 0.1 per cent was essential for these organisms.

Perotti (35) cultivated 10 Italian soils in a mannite solution and recorded the type of growth which varied from a thin scum, containing no cells typical of *Azotobacter*, to a heavy typical film.

Ashby (3) examined soil from 13 differently treated Rothamsted plots, only 4 of which yielded *Azotobacter*. Ashby also examined a number of South African "high veldt" soils in only one of which *Azotobacter* were noted. In the Rothamsted plots the presence of *Azotobacter* was limited to limed soils and was most abundant at the surface, though present 30 cm. deep.

One hundred samples of Java soil and water were examined for *Azotobacter chroococcum* by Kruyff (27), only 5 of which yielded this species. Kruyff states that other nitrogen-fixing forms were present but failed to indicate whether they belong to the *Azotobacter* group.

Greaves (23) quoted Feilitzen (15) as failing to observe *Azotobacter* in "Moorboden."

Wait and Squires (43) studied the vertical distribution of *Azotobacter* in alfalfa and corn field soil, and noted their presence at all depths down to 12 feet, except at the 1, 7, 10, and 11 foot levels in the former and at the 1, 3, 4, and 8 foot levels in the latter. This would indicate a rather wide vertical distribution in those particular soils.

*Azotobacter* were noted in 22 of 30 Colorado soils examined by Sackett (41). Among those failing to yield cultures of *Azotobacter* were a few non-cultivated and other cultivated soils taken from near the center of badly affected "niter spots." Sackett expressed the opinion that excessive nitrate content was responsible for the death of *Azotobacter* in "niter spots." Most of the soils examined were irrigated.

Lipman (28) reported a study of the vertical distribution of *Azotobacter* in 11 California soils, 4 of which contained no *Azotobacter*. When present they were found in all instances in both the first and second foot layers and in two instances as deep as 4 feet but no deeper.

Groenewege (24) secured markedly different results than Kruyff in examining soils from Java, in that *Azotobacter* were observed in all but one of a series (number unknown) examined. Their absence in this particular soil was believed due to the high (6.36 per cent) sodium chloride content.

A study of the seasonal distribution of *Azotobacter* by Peterson and Mohr (37), in which examinations were made of soil from the same plot each week for 39 consecutive weeks, showed good nitrogen-fixation throughout the entire period. *Azotobacter* were isolated every week except two. Three types were noted and isolated as follows: Type one, 31 times; type two, 6 times; and type three, 17 times during the 39 weeks.

An examination of 64 Danish forest soils by Weis and Bornebush (47)

revealed *Azotobacter* in only two instances. They were led to believe that the absence of these organisms was due to the lack of calcium carbonate, low temperature, and excessive humus.

Walton (46) reported *Azotobacter* from eleven localities in India but does not say whether these were all the soils examined. They were noted in some instances 2 to 3 feet below the surface.

Christensen (9) reported observations upon 145 soils, 47 per cent of which contained *Azotobacter*. A careful comparison of the presence of *Azotobacter* with calcium carbonate content and reaction was made. When litmus was used as the indicator 5 per cent of the acid, 14 per cent of the neutral, 60 per cent of the weakly alkaline, and 98 per cent of the strongly alkaline soils contained *Azotobacter*. When compared with the calcium carbonate content 33 per cent of those containing less than 0.1 per cent, 64 per cent of those containing 0.1 per cent to 0.2 per cent, and 100 per cent of those containing more than 0.2 per cent calcium carbonate were found to contain *Azotobacter*. It was also shown that of those soils which gave a response in crop yield to the application of lime only 3 per cent contained *Azotobacter*, compared with 89 per cent of those showing no response from lime.

Lipman and Burgess (29) examined about 50 soils that had been collected 15 to 20 years previous from a wide variety of conditions in numerous countries. A little more than one-third yielded cultures of *Azotobacter*; in all 20 cultures were isolated from 17 soils. The low per cent of soils containing *Azotobacter* might have been due to the long period of time the soils had been stored.

Omeliansky and Solonuskoff (34) examined soil from 26 widely separated localities in Russia and noted the presence of *Azotobacter* in only twelve instances from which they isolated 16 cultures. No effort was made to correlate the presence of *Azotobacter* with any soil characteristic.

*Azotobacter* were recorded as present in 8 soils examined by Canda (8) with very weak or no development from two subsoils tested. According to Canda the best tilled and ventilated soils, well provided with humus, and fertilized with minerals furnish the best conditions for these organisms to develop.

A study of 41 soils, many of which had been subjected to various fertilizer treatments, was reported by Christensen (10), only 15 of which contained *Azotobacter*.

Arndt (2) failed to detect *Azotobacter* in bog soil either before or one year after cultivation.

Thiele (42) reported upon the examination of soils from different elevations and observed a better distribution of *Azotobacter* in soils from low, below 1000 m., than from higher, above 1000 m. elevations.

Greaves (23) states that "*Azotobacter* are present in nearly all Utah soils."

Gainey (17) reported upon the examination of 90 soils, 52 of which contained *Azotobacter*. A comparison of the absolute reaction with the *Azotobacter* content showed that all soils, except three, that did not yield *Azotobacter* gave a pH of 5.9 or less while all those with a pH above 6.0 contained *Azotobacter* except three.

Unlimed cranberry soils were found to be free of *Azotobacter* by Waksman (44) while they were present in soils limed three years prior to the examination. The reaction of the unlimed soil was reported as pH 5.2 to 5.4 while the limed was pH 6.2 to 6.4.

Lipman and Waynick (30) noted the presence of *Azotobacter* in several samples of virtually barren calcareous sand from an island off the coast of Florida. There are a number of instances on record of these organisms being observed in sea water and along barren sea shores.

Nine of 17 soil types collected in eastern Ontario and examined by Jones and Murdock (26) contained *Azotobacter*.

Rushmann (40) studying the effect of continuous cropping upon the presence of *Azotobacter* in soils was led to the conclusion that some crops, especially legumes, were highly injurious to these organisms.

Ziemiacka (49) examined 28 soils from 13 stations in Poland and noted the presence of *Azotobacter* in 14. They seemed to be more abundant in soils with good moisture, humus, and calcium carbonate content and somewhat less abundant in soils rich in nitrogen.

Christensen (11) reported upon a study of 55 loamy and 40 sandy soils, in 57 of which *Azotobacter* were detected. Practically all neutral and alkaline soils regardless of type or location contained *Azotobacter* while they were present in very few acid soils.

Results secured from the study of 418 soils were reported by Gainey (18), 199 of which were found to contain *Azotobacter*. Of these soils 150 were local and 97 contained *Azotobacter*. One hundred and nineteen were collected in 37 other Kansas counties, 60 of which contained *Azotobacter*. The remaining 154 came from 24 states other than Kansas and only 42 of these gave *Azotobacter*. From this work the following conclusions were drawn:

"A definite and very close correlation has been established between the absolute reaction of the soil solution and the presence or absence of *Azotobacter* in the soil."

"Very few soils more acid than indicated by a hydrogen ion concentration of  $1 \times 10^{-6}$  contain *Azotobacter* while this group of organism is in most instances present in soils with a lower hydrogen ion concentration."

When these soils were divided into two groups with regard to reaction, more and less acid than represented by pH 6.0, and two groups with respect to the presence or absence of *Azotobacter*, and the association coefficient for reaction and *Azotobacter* determined it was found to be 0.96, or almost perfect.

Among the soils secured from other states there were 95 from eastern states, only 20 of which contained *Azotobacter*. The soils of this region are known to be strongly acid and these results indicate a much more limited distribution of this group of organisms there than in central or western soils. Even the application of lime was frequently not sufficient to induce an *Azotobacter* flora to develop. Many soils were examined to which insufficient lime had been added to render the reaction favorable. However, 24 limed soils were noted to which ample lime to correct the reaction had been added, yet did not contain *Azotobacter*. These soils, though, were from experimental liming plots located on large areas of very acid soils, and since the surrounding soils did not contain *Azotobacter* the chances for natural inoculation were very poor. In at least a dozen instances, though, it was quite evident that the application of lime had induced the development of an *Azotobacter* flora, because adjacent unlimed plots did not contain them. The following table illustrates the relationship observed between the reaction and the presence of *Azotobacter* in a group of 396 soils:

TABLE 1.—*The relation between reaction and the presence of Azotobacter*

Reaction	Number of soils	Azotobacter	
		Present	Absent
pH		per cent	per cent
Above 7.50	32	100	0
7.00 to 7.49	60	97	3
6.50 to 6.99	45	90	10
6.00 to 6.49	47	80	20
5.50 to 5.99	122	20	80
5.00 to 5.49	43	14	86
4.50 to 4.99	44	7	93
Below 4.50	3	0	100

Yamagata and Itano (48) studied the distribution of *A. chroococcum*, *A. beijerinck*, and *A. vinelandii* in 300 Japanese soils. The soils were arranged in four groups depending upon their reaction and in Table 2 the distribution of the three species in soils of various reactions are shown:

TABLE 2.—*The distribution of three bacterial species in soils of various reactions (Yamagata and Itano)*

Reaction	Number of soils examined	Number of soils containing the different species		
		<i>A. chroococcum</i>	<i>A. beijerinck</i>	<i>A. vinelandii</i>
Acid	119	0	0	0
Neutral	76	3	11	0
Slightly alkaline	62	26	20	0
Alkaline	43	37	0	3

Christensen (12) reported by far the most extensive study of the distribution of *Azotobacter* thus far recorded. A comparison of the hydrogen ion concentration of the soil solution was determined colorimetrically and the presence of *Azotobacter* in 3161 soils is given. This group of organisms was not detected in 1806 or 57 per cent of the soils examined. The following data are taken from Christensen's paper:

TABLE 3.—*The distribution of Azotobacter in soils of various reactions (Christensen)*

Reaction	Number of soils	Not containing <i>Azotobacter</i>	Containing <i>Azotobacter</i>
pH		per cent	per cent
5.8 or less	180	100	0
6.0	479	91.8	8.2
6.2	673	71	29
6.4	765	40	60
6.6	499	6	94
6.8	398	0.3	99.7
7.0 or above	248	0	100

Asō and Yamagata (4) reporting upon the examination of a group of 300 soils showed that *Azotobacter* are much more widely distributed in certain parts of Japan than in others, and express the opinion that the climate is an important factor in governing their distribution. However, the evident correlation with reaction would indicate that the relative infrequency of *Azotobacter* in certain areas is more likely due to the influence of some climatic factor or origin of soil upon the soil reaction rather than directly to climate or location. The group of organisms were apparently as widely distributed in rice field soils as in other cultivated soils.

In studying the effect of various fertilizer treatments of the soil Duggeli (14) concluded that plowing and fertilizing with potash and phosphorus increased the number of *Azotobacter* in soils. The numbers were also observed to decrease from April to July, probably because of lowered moisture content and increase in protozoa.

Brenner (6) reported upon the examination of 200 soils of Finland and found *Azotobacter* to be rare even in cultivated soils, due, he believed, to high acidity and a low buffering effect. Many soils, including peat, swamp, some clays rich in electrolytes, and iron bearing strata of podsol soils, were unfavorable for *Azotobacter* even after being limed.

Petri (38) reported a case in which *Azotobacter* were less abundant in cultivated than non-cultivated soil, and expressed the opinion that the drying out following cultivation might be the injurious factor. In neither soil were they found at a depth of 50 cm.

In an extended study of the successive forms of life developing in areas destroyed by the lava flows of Vesuvius, Rossi and Riccardo (39) noted

Azotobacter in samples of soil taken from 4 out of 5 locations including the lava of 1906. Near the sea level, Azotobacter were found throughout the year, whereas at 500 to 800 m. above sea level, even when present, were only observed during winter months.

A comparison of the pH values of 641 soils determined electrometrically and the presence of Azotobacter was reported by Christensen and Jensen (13). Azotobacter were not detected in 336 or 52 per cent of the soils. The following condensation of their data (Table 4) illustrates the frequency with which Azotobacter were encountered in soils of various reactions:

TABLE 4.—*The occurrence of Azotobacter in soils of various reactions (Christensen and Jensen)*

Reaction	Number of soils	Not containing Azotobacter	Containing Azotobacter
pH		per cent	per cent
5.8 or less	57	100	0
5.8 to 6.0	40	95	5
6.0 to 6.2	66	89.4	10.6
6.2 to 6.4	58	86.8	13.2
6.4 to 6.6	74	81.1	18.9
6.6 to 6.8	75	60	49
6.8 to 7.0	36	25	75
7.0 to 7.2	52	13.5	86.5
7.2 to 7.4	32	6.2	93.8
7.4 or above	141	0	100

These data vary from the colorimetrically determined pH comparisons previously recorded only in that Azotobacter seemed to be slightly more sensitive to acid in this series or perhaps the soils give a slightly higher pH by the electrometric method than by the colorimetric method.

Niemeyer (32) noted Azotobacter in 54 per cent of all soils examined. They were found in 74 per cent of surface cultivated soils. No soil more acid than pH 5.5 contained them. Drainage water, except from strongly acid soils, also contains these organisms. This investigator believed that reaction and aeration were the predominant factors determining the presence of Azotobacter in soils.

Studying the effect of soil reaction upon Azotobacter, Petersen (36) observed few soils appreciably below pH 6.0 that contained these organisms and none more acid than pH 5.5 in which they were present. They were found to retain their viability for some time at pH 5.8. It was suggested that a high nitrate nitrogen content may cause other organisms to overshadow Azotobacter.

Gibbs (22) examined 106 timber soils of Idaho and noted the presence of Azotobacter in only 24. They were found to a slightly greater extent in



virgin timber soils than in soils under cultivation. All soils, however, were capable of supporting *Azotobacter* for some time if they were introduced. All but 14 of 60 tested still contained *Azotobacter* after 15 to 18 months. Gibbs believes tree products are toxic to *Azotobacter*. No correlation was noted between reaction and distribution of *Azotobacter*; however, there were only three soils recorded with a hydrogen ion concentration greater than  $1 \times 10^{-6}$  or more acid than these organisms are known to tolerate. Thirty-five soils were recorded as having a pH between 7.1 and 8.0, and 27 between pH 8.1 and 9.2, yet it is stated that all soils reacted acid to the Truog and Veach tests, two facts that appear difficult to correlate and would tend, in the face of so much data contrary to the findings of Gibbs, to indicate that possibly some error existed in the hydrogen ion concentration determinations.

Abbott (1) noted the presence of *Azotobacter* in cane but absent from cotton soils.

Loew (31) recorded *Azotobacter* in certain Brazilian and Porto Rican soils said to be slightly acid and free from calcium carbonate.

A study of 497 soils was reported by Niklas and Poschenrieder (33), one-third of which contained *Azotobacter*. A comparison of the reaction with the presence of *Azotobacter* showed that as the acidity increased the per cent of soils free from *Azotobacter* increased from 47 per cent at pH greater than 7.0 to 100 per cent at pH less than 4.5. Only 20 per cent of the soils more acid than pH 6.0 gave moderate to strong *Azotobacter* development while 72 per cent less acid than pH 6.0 gave similar development.

Some correlation was also found between the phosphorus content and the presence of *Azotobacter*; a high phosphorus content being associated with a wider distribution of *Azotobacter*. The opinion was expressed that while the reaction must be regarded as the principle controlling factor, the phosphorus content should not be overlooked.

## SUMMARY

In drawing conclusions relative to the distribution of *Azotobacter* in soils there are certain pertinent facts that should be kept in mind.

In the first place, all knowledge relative to this group of organisms has been accumulated during the past quarter of a century. Methods of investigation are still in the chaotic condition common to all newly opened fields of research. In fact, the variations in methods of testing a soil for the presence of *Azotobacter* are almost as numerous as the number of investigators. Future research may show that none of the methods now in vogue are satisfactory or even of value. Certainly the methods now employed are, at best, only relative and not absolute. Evidence has been noted that would indicate soils containing a relatively small number of *Azotobacter* may be reported as negative by such methods as have been employed in most of the investigations to which attention has been called.

Furthermore, it is rather difficult to maintain aseptic conditions in handling soils and it is probable that in some instances the necessary precautions have not been taken to prevent infection with *Azotobacter*, either during or subsequent to sampling.

Finally soil biologists are not entirely agreed as to just what characteristics an organism must possess to be included among the *Azotobacter*. In few instances have pure cultures been isolated and identified, the investigator relying upon the type of film developed or, at best, resorting to a superficial microscopic examination. This has almost certainly lead to erroneous conclusions in some instances.

In spite of these difficulties it is believed that in most instances where *Azotobacter* have been reported absent they were either absent or present in such small numbers as to be of little significance. Also, that soils reported as containing these organisms probably contained them in appreciable numbers.

The above assumptions being true, *Azotobacter* are certainly widely distributed over the earth's surface, having been noted in practically every locality from which soils have been examined. They have been recorded in both extremely hot and cold regions; where rainfall is very heavy and where practically absent; in fertile soil, barren sand and newly formed volcanic soil; at high as well as low elevations; in surface and subsoil; in drainage and in sea water; in forest, meadow and cultivated soils.

Nevertheless, approximately half of all soils examined have failed to show the presence of *Azotobacter*. This raises the very important question as to what factor or factors control the distribution of these organisms in soils.

Attention has been called to numerous factors suggested to explain the failure of *Azotobacter* to develop in certain soils. Many of these are probably contributory or possibly of primary importance in limiting development under certain conditions. Most of them, however, cannot be of sufficiently wide application to account for the absence of *Azotobacter* in half the earth's surface.

There is one factor, however, to which attention has been called that would seem to have sufficiently wide application to account for the presence or absence of *Azotobacter* in practically all soils; i.e., the reaction of the soil. Numerous physiological investigators, such as reported by Gainey and Batchelor, have established beyond question the sensitiveness of this group of organisms to an acid condition. Almost without exception *Azotobacter* have been found absent from strongly acid and present in neutral or slightly alkaline soils. Whether their absence is due directly to the hydrogen ion concentration or to some secondary change induced by the hydrogen ion concentration has not been definitely established and is immaterial to the present discussion, The fact remains

that a markedly acid soil condition and a vigorous *Azotobacter* flora are incompatible.

There is some discrepancy relative to the absolute hydrogen ion concentration tolerated by *Azotobacter*. This is not surprising, though, if the fact be kept in mind that the methods of determining the absolute reaction of a soil are still in the experimental stage. In fact the same investigator using two or more methods does not arrive at the same end point of tolerance. Furthermore, it would be surprising if all the members of a large group of organisms, such as *Azotobacter*, exhibited the same hydrogen ion tolerance. It is possible that different investigators have been dealing with organisms possessing different degrees of sensitiveness to hydrogen ions. In general, the limit of tolerance appears to be somewhere near an hydrogen ion concentration of  $1 \times 10^{-6}$ . Undoubtedly future investigations will clarify many points now unexplained.

Not only is there a close correlation between the soil reaction and the natural distribution of *Azotobacter* but it has been shown by Gainey (19, 20, 21) that the ability of a soil to support these organisms can be controlled at will, merely by artificially adjusting the reaction.

#### LITERATURE CITED

- (1) Abbott, E. V. 1926. Louisiana. Agr. Expt. Sta. Bull. 194, 1.
- (2) Arndt, T. 1917. Mitt. Ver. Förd. Moorkult. Deut. Reiche. 35 p. 269. (Bact. Abst. 3: 149).
- (3) Ashby, S. F. 1907. Jour. Agr. Sci. 2: 35.
- (4) Asö, K. and Yamagata, U. 1924. Actes 4th Confer. Internat. Pedologie. III, p. 192.
- (5) Beijerinck, M. W. 1901. Centbl. Bakt. [etc.]. 7: 561.
- (6) Brenner, W. 1924. Geol. Komm. Finland Agroeol. Meddel. 20, p. 15 (Expt. Sta. Rec. 55: 813).
- (7) Burri, R. 1904. Schweiz. Ztschr. Forstw. 55, p. 89 (Principles of Soil Microbiology. p. 115).
- (8) Canda, A. 1916. Staz. Sper. Agr. Ital. 49: 125. (Internatl. Rev. Sci. and Pract. Agri. 7: 801).
- (9) Christensen, H. R. 1915. Centbl. Bakt. [etc.]. 43: 1.
- (10) ———. 1917. Ibid. 17: 109.
- (11) ———. 1923. Soil Sci. 15: 329.
- (12) ———. 1923. Sonderabdruck aus: Internatl. Mitt. Bodenk. 13: 1.
- (13) ———, and Jensen. 1924. Ibid. 14: 1.
- (14) Duggeli, M. 1924. Landw., Jahrb. Schweiz. 38: 203. (Expt. Sta. Rec. 52: 720.)
- (15) Feilitzen, H. von. 1910. Fühling's Landw., Ztg. 59: 489.
- (16) Fischer, H. 1906. Centbl. Bakt. [etc.]. 15: 235.
- (17) Gainey, P. L. 1918. Jour. Agr. Research [U. S.]. 14: 265.
- (18) ———. 1923. Ibid. 907.
- (19) ———. 1923. Ibid. 24: 289.
- (20) ———. 1925. Actes 4th Confer. Internat. Pedologie, III. p. 31.
- (21) ———. 1925. Soil Sci. 20: 73.
- (22) Gibbs, Wm. 1925. Idaho Agr. Exp. Sta. Bull. 135.
- (23) Greaves, J. E. 1918. Soil Sci. 6: 163.

- (24) Groenewege, J. 1913. Arch. Suikerind. [U. S.] 21: 790. (Chem. Abst. 7: 3635.)
- (25) Heinze, B. 1904. Centbl. Bakt. [etc.]. Abt. 2, Bd. 12: 43.
- (26) Jones, D. H. and Murdock, F. G. 1919. Soil Sci. 8: 259.
- (27) Kruyff, E. de. 1910. Centbl. Bakt. [etc.]. Abt. 2, Bd. 26: 54.
- (28) Lipman, C. B. 1912. Univ. Cal. Pub. Agr. Sci. 1: 1.
- (29) ———, and Burgess, P. S. 1915. Centbl. Bakt. [etc.]. Abt. 2, Bd. 44: 481.
- (30) ———, and Waynick, D. D. 1918. Natl. Acad. Sci. Proc. 4: 232.
- (31) Loew, O. 1927. Centbl. Bakt. [etc.]. Abt. 2 Bd. 70: 36.
- (32) Niemeyer, L. 1924. Bot. Arch. 7: 347. (Expt. Sta. Rec. 55: 720.)
- (33) Niklas, H. and Paschenrieder, H. 1927. Centbl. Bakt. [etc.]. Abt. 2, Bd. 71: 251.
- (34) Omeliansky, V. L. and Solounskoff, M. 1915. Extrait des: Arch. Sci. Biol. Petrograd, 18: No. 5: 1.
- (35) Perotti, R. 1906. Rendiconti della: R. Accad. Lincei. Com. Sci. Aliment. [Rome] Pub. 15: 295.
- (36) Petersen, E. J. 1925. Tidsskr. Planteavl. 31: 246, (Exp. Sta. Rec. 55: 721).
- (37) Peterson, E. G. and Mohr, E. 1913. Centbl. Bakt. [etc.]. Abt. 2, Bd. 38: 494.
- (38) Petri, L. 1924. Actes 4th Confer. Internatl. Pedologie. III, p. 246.
- (39) Rossi, G. and Riccardo, S. 1924. Actes 4th Confer. Internatl. Pedologie. III. p. 115.
- (40) Ruschmann. 1920. Mitt. Biol. Reichsanst. Land. u. Forstw. 18; 159. (Centbl. Bakt. [etc.]. Abt. 2, Bd. 56: 132.)
- (41) Sackett, W. G. 1911. Colorado Agri. Expt. Sta. Bull. 179.
- (42) Thiele, R. 1918. Landw., Vers. Sta. 63, p. 161.
- (43) Wait, H. H. and Squires, D. H. 1910. Nebraska Agric. Expt. Sta. Ann. Rpt. 1910, p. 160.
- (44) Waksman, S. A. 1918. Science n. s. 48: 653.
- (45) ———. 1927. Principles of Soil Microbiology, p. 115.
- (46) Walton, J. H. 1915. India Dept. Agr. Mem., Bact. Ser. 1: 95.
- (47) Weis, Fr. and Bomebush, C. H. 1914. Forstl. Forsøgsv. Danmark. 14: 319. (Internatl. Rev. Sci. and Pract. Agri. [Rome]. 6: 546.)
- (48) Yamagata, U. and Itano, A. 1923. Jour. Bact. 8: 521.
- (49) Ziemieka, E. 1922. Compt. Rend. Confer. Extraordinaire (3rd Internatl.) Agropediologique, Prague, 1922, p. 206.

# ÜBER STICKSTOFFBINDUNG DURCH FREI LEBENDE MIKROORGANISMEN IM BODEN

W. BRENNER

*Helsingfors, Finland*

Wenn auch der grösste Teil der Stickstoffbeträge, die an einem gegebenen Orte von den Pflanzen aufgenommen werden, nach längerer oder kürzerer Zeit wieder dem Boden und neuen Pflanzen zugute kommen, lehrt eine einfache Überlegung, dass bedeutende Verluste nicht zu vermeiden sind. Man braucht nicht nur an die Denitrifizierung oder richtiger die Stickstoffentbindung zu denken, deren Bedeutung in der Natur noch nicht aufgeklärt ist. Auch die Nitrifizierung, die in allen besseren, nicht zu sauren Böden stattzufinden scheint, bringt grosse Verluste mit sich. Die Nitrate werden bekanntlich nicht adsorbiert, sondern in dem Falle, dass sie nicht direkt von den Pflanzen ausgenutzt werden, vom Regenwasser wegtransportiert, was in den nordischen Ländern besonders im Frühling und Herbst in nicht zu unterschätzender Ausdehnung vor sich geht. Noch ist daran zu erinnern, dass durch die Bildung und Anhäufung von organischem Substanz, z. B. Torf, grosse Mengen von Stickstoff auf längere Zeit gebunden und somit dem Kreislaufe entrissen werden. Für Kulturböden kommt natürlich ausserdem der mit den Ernten weggeschleppte Stickstoff in Betracht.

Durch welche Vorgänge werden nun diese sicher sehr verbreiteten und bedeutenden Verluste gedeckt? Die mit dem Regen aus der Atmosphäre mitgebrachten N-Verbindungen genügen sicher nicht. Es bleibt uns nur die mikrobiologische Stickstoffbindung übrig in bezug auf Böden, die nicht künstlich gedüngt werden.

Aussero dentlich wichtig ist die viel studierte Bindung, die durch in Symbiose mit höheren Pflanzen vor allem Leguminosen lebenden Mikroorganismen zustande kommt. Aber Leguminosen wachsen nicht an jedem Orte und die in ihren Wurzelknöllchen tätigen Bakterien können nach den neuesten Untersuchungen von Barthel nicht allein, wenigstens nicht in Reinkultur Stickstoff fixieren. Will man also die überall im Boden stattfindenden Verluste gedeckt sehen, so muss man nach anderen, verbreiteten, in den Böden frei lebenden, fixierenden Mikroorganismen suchen.

Es ist sehr wahrscheinlich, dass die nützliche Fähigkeit Stickstoff zu binden unter den Bodenmikroorganismen ziemlich allgemein ist. Es sind auch eine grosse Zahl solcher Organismen meist Bakterien bekannt und

mehr oder weniger eingehend untersucht worden. Die wichtigste und am meisten studierte Form ist ohne Zweifel der im Jahre 1901 von Beijerinck entdeckte *Azotobacter chroococcum*, dem gewöhnlich die häufig in Böden verschiedener Art beobachteten N-Gewinne zugeschrieben werden.

Meine eigenen Untersuchungen hatten auch anfangs den Zweck das Vorkommen und die Bedeutung von *Azotobacter* in den finnländischen Böden zu studieren. Es zeigte sich aber bald, dass dieser Organismus sehr selten war. Von 200 Bodenproben, etwa die Hälfte natürliche, die andere Hälfte Kulturböden aus verschiedenen Teilen Finnlands, die ich mit Hilfe von Beijerincks Mannit-lösung prüfte, entwickelten nur 2 *Azotobacter*. Diese zwei waren besonders gut gepflegte Garten- und Acker-Böden mit neutraler Reaktion.

Bekanntlich ist das Vorkommen von *Azotobacter* schon früh in Zusammenhang mit dem Gehalt des Bodens an Kalk oder basischen Stoffen überhaupt gebracht worden. Jetzt wissen wir dass dieser Organismus sehr von der H-Ionenkonzentration abhängig ist. Als Regel gilt nach Bondorff und Christensen, dass dieses Bakterium, wenn es auch ausnahmsweise noch bei pH 6,0–5,8 wachsen kann, für sein gutes Cedeihen und einigermaßen regelmässiges Vorkommen eine beständige Reaktion von pH 6,7 oder darüber verlangt.

Diese Forderungen erfüllen nun die finnländischen Böden in den allermeisten Fällen nicht. Von 815 untersuchten finnländischen Kulturböden lagen nicht weniger als etwa 92% in bezug auf ihre Reaktion unterhalb der gewöhnlich angenommenen *Azotobacter*-Grenze. Die natürlichen Böden bieten noch ungrünstigere Bedingungen, denn die obersten Schichten sind infolge der Humus- und Torfbildung oft sogar sehr sauer und der darunter liegende silikatische Mineralboden hat gewöhnlich eine zu unbeständige Reaktion. Das Fehlen von *Azotobacter* in den finnländischen Böden bekommt durch diese Tatsachen eine natürliche Erklärung.

Ausser der Reaktion können noch andere Faktoren in der Natur schädlich auf *Azotobacter* einwirken. In den meisten Böden konnte zwar nach Zusatz von  $\text{CaCO}_3$  und Impfung aus einer kräftigen Rohkultur *Azotobacter*vegetation erzielt werden. Interessant ist aber dass Bodenproben aus den Anreicherungsschichten der Podsolprofile auch mit  $\text{CaCO}_3$  keine *Azotobacter*-Vegetation ergaben. Dagegen wuchs unser Bakterium nach Kalkzusatz sowohl in der Rohhumusschicht als in der Bleicherde und dem Untergrund desselben Profils ausgezeichnet. Die gewöhnliche Rosterde enthält somit offenbar unabhängig von der Reaktion für *Azotobacter* schädliche Stoffe.

Es ist also erwiesen dass *Azotobacter* für die N-Bindung in finnländischen Böden keine Rolle spielen kann und dasselbe wird auch für übrige karbonatarme Urgesteinsgebiete mit humidem Klima und dominierenden Podsolböden gelten. Um die auch hier auftretenden N-Gewinne im

Boden erklären zu können muss man sich an andere Mikroorganismen wenden.

Die zur *Amylobacter*-Gruppe gehörenden Bakterien *Clostridium* u. a. sind auch in sauren Böden sehr verbreitet und nehmen sicher an der N-Bindung teil. Ihre mehr oder weniger ausgeprägte anaerobe Lebensweise bringt aber mit sich, dass sie nicht in gut durchlüfteten Böden gedeihen und somit entgegengesetzte Ansprüche gegenüber den höheren Pflanzen aufweisen. Hierdurch dürfte ihre Bedeutung für die Vegetation erheblich vermindert werden.

Mit den bis jetztgenannten Bakterien sind aber die N-bindenden Mikroorganismen keineswegs erschöpft. Man kennt noch viele Bakterienarten trivialer Typus, die diese Fähigkeit besitzen, und vor allen kommen einige niedere Pilze meist "Fungi imperfecti" besonders für saure Böden in Betracht. Wenn z. B. Henry in Waldböden eine N-Zunahme von bis 13 kg. pro Hektar und Jahr erhielt, ist dieser Betrag wohl in erster Linie auf die Tätigkeit der humusbewohnenden Schimmelpilze zu schreiben.

In den Böden, die nicht allzu sauer sind leben diese Schimmelpilze zusammen mit anderen Mikroorganismen Actinomyceten, Hefen und Bakterien komplizierte Mikroorganismengesellschaften bildend. Diese können, wie wir weiter sehen werden, höchst bedeutende N-Mengen binden. Es kann aber sehr schwierig sein diese Gesellschaften zu analysieren und zu entscheiden welcher oder welche von den Symbionten die eigentlichen N-Sammler sind. Aus ihrem natürlichen Zusammenleben isoliert können sie entweder ihre nützliche Tätigkeit ganz einstellen oder doch sehr herabsetzen.

Wenn man sich eine Vorstellung von der Grösse der N-Bindung verschaffen will, die in verschiedenen Böden stattfinden kann, hat es auch kein Zweck solche Isolierungen vorzunehmen. Im Gegenteil arbeitet man viel vorteilhafter mit Rohkulturen, wo die natürliche Zusammensetzung der Gesellschaften möglichst unbeeinflusst bleibt. So wurde auch bei meinen Untersuchungen vorgegangen.

Die Beijerinck-schen Mannitlösungen, die mit kleinen Mengen Erde geimpft worden waren, und wo sich *Azotobacter* nicht entwickelte, blieben aber nicht steril. In einigen entwickelte sich *Amylobacter* und eine makroskopisch sichtbare Vegetation blieb aus. In anderen entwickelte sich nur kümmerliches Mycel, aber in einer grossen Zahl der Versuchskolben kamen so kräftige Vegetationen zustande, dass es undenkbar war, sie hätten mit den kleinen N-Mengen der Impferde ausgekommen. Anfangs schienen in diesen Kulturen die Bakterien bisweilen auch Hefen zu dominieren, dann nahmen die Schimmelpilze Überhand so dass die Kulturflüssigkeit nach 2-3 Wochen bei Zimmertemperatur mit einem Filz aus Pilzhäuten bisweilen dunkelbräunlicher, bisweilen gelblicher Farbe bedeckt war.

Einige solche Lösungskulturen die 0,5 g. Mannit auf 25 cc. enthielten

und mit 5 cc. Bodenemulsion geimpft waren gaben nach 3 Monate bei Zimmertemperatur mit und ohne  $\text{CaCO}_3$  folgende N-Gewinne:

*TABELLE 1.—Stickstoff-Gewinne nach 3 Monate bei Lösungs Kulturen die 0,5 g. Mannit auf 25 cc. enthielten und mit 5 cc. Bodenemulsion geimpft waren*

Ort	Bodenart	Mg N gefunden in Kultur		Mg N in Blinde Probe
		ohne $\text{CaCO}_3$	mit $\text{CaCO}_3$	
Tervajoki	Leichter Ton	0,6	0,6	0,6
Tammerfors	Steifer Do	4,0	4,5	0,6
Kyrkslätt	Do Do	4,0		1,2
Do	Do Do	3,5		0,8
Valkeala	Mull	3,0	6,0	0,6
Do	Do	3,7	7,0	
Inga Teira	Do	4,0	4,0	0,8
Do	Do	5,0	5,5	
Do Brannbolsta	Steifer Ton	0,9	4,5	0,8
Borga, Storgard I	Leichter Do	3,0	3,0	0,6
Do Do II	Steifer Ton	3,5	4,5	0,7
Do Do II	Do	4,0	6,0	
Do Kullo I	Do	3,0	4,0	0,7
Do Do I	Do	2,5	5,0	0,7
Do II	Sand	2,5	3,5	0,7
Do II	Do	2,7	4,5	0,7
Do II	Do	3,0	4,5	

Die in der obigen Tabelle aufgenommenen Böden waren sämtliche Kulturböden aus verschiedenen Teilen Finnlands.

Aus den mit Erde versetzten kräftigen Kulturen konnten die Vegetationen in praktisch N-freien Mannitlösungen geimpft werden. Das Wachstum war viel langsamer; auch kamen hier nicht so regelmässig gute Pilzdecken zustande. Dagegen gediehen die Vegetationen vorzüglich in Röhren mit praktisch N-freiem Mannit-Agar. Auf diesem Substrate habe ich sie bei jährlicher Überimpfung bis 4 Jahre lebend erhalten können.

Der Beweis, dass diese Mikroorganismengesellschaften wirklich ihren N-Bedarf aus dem atmosphärischen, freien Stickstoff nehmen bekommen man aber erst, wenn solche Kulturbedingungen vorhanden sind, die den Zutritt von Stickstoffoxyde und Ammoniak aus der Luft verhindern. Bei den entscheidenden Versuchen wurden deshalb die Kulturkolben unter dicht schliessenden tubulierten Glasglocken gestellt, in deren Mündungen Röhre mit Natronkalk und in Schwefelsäure getauchten Bimsteinstückchen angebracht waren, oder auch wurden die Mündungen der Versuchskolben selbst mit solchen Röhren versehen. Dann und wann wurde ein langsamer Luftstrom durchgesaugt. Die Kulturgefässe waren teils kleinere mit 25 cc., teils grössere mit 100 cc. 2%-ige Mannitlösung beschickte Erlenmeyerkolben. Zwei Azotobacter enthaltende Böden wurden zum Vergleich herangezogen.



Die Kulturen mit 25 cc. Lösung enthielten nach 3 Monate folgende N-Mengen:

*TABELLE 2.—Stickstoff-Gewinne nach 3 Monate bei Kulturen von Mikroorganismengesellschaften (25 cc. 2 %-ige Mannillösung) woran der Zutritt von Stickstoffoxyde und Ammoniak aus der Luft Verhindert war*

Ort	Bodenart oder Kulturboden	Mg N gefunden in Kultur		Mg N in Blinde Probe
		ohne CaCO <sub>3</sub>	mit CaCO <sub>3</sub>	
Juuka I	Waldhumus	1,2	1,7	
Do II	Do	1,9	2,5	
Puolanka	Do	1,0	1,6	
Ingå	Do		1,5	
Borgå	Do		1,8	
Juuka III	Waldtorf	1,4	2,5	
Elimå	Do	0,6	0,5	
Kaavi I	Wiesenumus		1,6	
Do II	Braunmoostorf	0,8	2,5	
Do III	Do		1,7	
Do IV	Riedgrastorf	1,4	1,7	
Elimå	Do	0,4	0,5	
Limingo	Do		1,5	
Valkeala	Mull	0,3	1,5	0,2
Ingå	Do	0,7	0,5	
Borgå	Steifer Ton	0,8	1,3	
Tammerfors (Azotobacter)	Do	1,7	2,5	
Helsingfors (Azotobacter)	Mull		2,1	0,1

Beim Impfen der grösseren 100 cc. Lösung enthaltenden Kolben wurde teils Mycel aus Agarkulturen, teils Bodenemulsionen verwendet. Das Resultat war folgendes:

*TABELLE 3.—Stickstoff-Gewinne nach 3 Monate bei Kulturen von Mikroorganismengesellschaften (100 cc. 2 %-ige Mannillösung) woran der Zutritt von Stickstoffoxyde und Ammoniak aus der Luft verhindert war*

Ort	Bodenart	Mg N gefunden in Kultur	Mg N in Blinde Probe
		mit CaCO <sub>3</sub>	
Juuka	Impfmaterial: Agarkultur		
Valkeala	Waldhumus	4,5	
Helsingfors (Azotobacter)	Ackermull	6,7	
	Gartenmull	7,1	0,4
Loimaa	Impfmaterial: frische Erde.		
	Ackermull	6,7	0,3
Träskända	Do	6,2	0,2
Helsingfors (Azotobacter)	Gartenmull	6,7	0,4

Auf Mannit-Agarplatten, ebenfalls unter Glocke gestellt, wurden die N-Gewinne kleiner, wahrscheinlich weil eine vollständige Ausnützung der Nahrung hier nicht stattfinden konnte. Folgende N-Beträge wurden in 25 cc. umfassenden 2% Mannit enthaltende Platten gefunden:

*TABELLE 4.—Stickstoff-Gewinne nach 3 Monate bei Kulturen von Mikroorganismengesellschaften (25 cc. umfassenden 2% Mannit enthaltenden Agarplatten) woran der Zutritt von Stickstoffoxyde und Ammoniak aus der Luft verhandertwar*

Ort	Bodenart	Mg N gefunden in Kultur	Mg N in Blinde Probe
		mit CaCO <sub>3</sub>	
	Natürliche Böden		
Juuka I	Waldhumus	1,3	
Do II	Do	1,0	
Do III	Do	0,8	
Do IV	Niedermoortorf	1,5	
	Kulturböden		
Valkeala	Mull	0,9	
Ingå, Teira	Do	0,8	
Borgå, Storgard	Steifer Ton	0,9	
Do Kullo I	Do	0,6	
Do Do II	Sand	0,8	
Tammerfors (Azotobacter)	Steifer Ton	2,2	0,1

Durch die obigen Versuche ist also einwandfrei gezeigt worden, dass auch Böden, in welchen Azotobacter nicht vorhanden ist, und zwar sowohl natürliche als Kulturböden, kräftig N-fixierende Mikroorganismengesellschaften enthalten können. Die gebundenen N-Mengen waren meist deutlich kleiner als die mit Azotobacter erhaltenen; in einigen Fällen können sie aber gut mit diesen wetteifern. In den Kulturen mit 100 cc. Lösung war der grösste gebundene N-Betrag (wenn die N-Menge der blinden Probe abgezogen wird) 3,2 mg. N auf 1 g. zur Gebote stehenden Mannit.<sup>1</sup> In den 25 cc. umfassenden Kulturen wurde bis 4,6 mg. N. auf 1 g., Mannit gebunden. Die entsprechenden Maximiwerte waren für die Azotobacter-Vegetation 3,4 bzw. 4,8 mg. Aus den Tabellen geht weiter hervor dass die Anwesenheit von CaCO<sub>3</sub> die N-Bindung meist kräftig gefördert hat.

Was sind es nun für Böden, die solche N-fixierende Vegetationen geliefert haben? Betrachten wir erst die natürlichen Böden.

Die untersuchten Bodenproben in einer Zahl von 100 stammen aus verschiedenen Teilen Finnlands und aus verschiedenen Pflanzengesellschaften. Gute Vegetationen gaben Mullböden aus edlen Laubwäldern sowie aus krautreichen Erlen- und Fichtenwäldern, weiter Torfböden aus

<sup>1</sup>Es wurde nicht untersucht ob der Mannit gänzlich verbraucht wurde.

Braunmoos-Niedermooren und anderen kalkliebenden Moorgesellschaften. Auch aus dem Torf der gewöhnlichen Seggensümpfe konnten gute, ziemlich reine Pilzvegetationen erhalten werden. Dagegen fielen die Versuche mit dem Rohhumus aus den gewöhnlichen moosreichen Nadelwäldern (Kiefer und Fichte) sowie mit dem *Sphagnum fuscum*-Torfe aus den Hochmooren negativ oder fast negativ aus und zwar einerlei ob  $\text{CaCO}_3$  anwesend war oder nicht.

Man kann also behaupten dass die N-bindenden Vegetationen auch in natürlichen Böden nicht selten sind. Sie treten hier besonders in solchen Pflanzengesellschaften auf, wo erfahrungsgemäss eine intensive Nitrifizierung vor sich geht und die N-Verluste folglich auch gross werden können.

Die zweite Hälfte der untersuchten Bodenproben bezog sich auf Kulturböden. Von diesen gaben im allgemeinen die mehr oder weniger humushaltigen Mineralböden (Sand und Ton) aus den südlichen Teilen Finnlands gute Vegetationen. Ausnahmen waren leichte, saure Tonböden. Im westlichen Finnland an der Küste des Bottnischen Meerbusens dominieren solche Tonböden und aus diesen Gegenden wurden auch sehr unregelmässig und hauptsächlich mit weniger sauren Böden sicher positive Ergebnisse erzielt.

Die fraglichen N-Sammler scheinen also nicht gleichgültig für die Reaktion zu sein. Dass sie nicht neutrale oder schwach alkalische Reaktion meiden lehrt schon die Tatsache, dass sie gut in der schwach alkalischen Beijerinck-schen Lösung gedeihen und sogar durch  $\text{CaCO}_3$ -Zugabe gefördert werden. Die Böden, aus welchen gute Vegetationen erhalten wurden, waren meist mässig sauer, d.h. ihre Reaktion fiel in den meisten Fällen zwischen den pH-Grenzen 5,5–6,5. Saurere Böden enthielten dagegen sehr unregelmässig N-bindende Gesellschaften dieses Typus. Zwei neutrale Böden (Helsingfors und Tammerfors) gaben *Azotobacter*.

Wenn also die *Azotobacter*-Vegetation der N-Sammler der neutralen Böden ist, können die hier behandelten Gesellschaften, die ich vorläufig nur als *Pilz-Bakterien*-Vegetation bezeichnen möchte, als die Sammler der mässig sauren Böden gelten.

Die N-fixierenden Organismen der extrem sauren Böden (wahrscheinlich wird es auch solche geben) sind aber noch zu entdecken. Für sie kann kaum die Beijerinck-schen Lösung wegen ihrer schwach alkalischen Reaktion geeignet sein. Ich habe einige Versuche gemacht eine solche Mannitlösung mittels Phosphat und Phosphorsäure auf pH 5 zu stabilisieren und dann mit sauren Böden zu beimpfen. Das Ergebnis war nicht besser als mit der ursprünglichen Lösung. Es müssen also in der Zukunft noch andere Medien versucht werden.

# DIE STICKSTOFFBINDUNG DURCH BAKTERIEN

C. STAPP

*Biologische Reichsanstalt für Land- und Forstwirtschaft, Berlin, Deutschland*

## EINLEITUNG

Wenn wir rückschauend Betrachtungen anstellen über die Erfolge landwirtschaftlich- und bodenbakteriologischer Forschung, so erscheint uns als eines der wichtigsten Ergebnisse in dieser Richtung die Entdeckung und Diagnostizierung derjenigen Gruppen von Bakterien, denen die Fähigkeit zukommt, den freien atmosphärischen Stickstoff zu assimilieren, d.h. ihn chemisch zu binden und ihn damit mittel- oder unmittelbar der landwirtschaftlichen Produktion nutzbar zu machen.

Auf die historische Entwicklung, wie man aus anfänglichen Beobachtungen, nach denen sich der Boden scheinbar selbsttätig mit Stickstoffverbindungen anreicherte, schliesslich zur Erkennung von Microorganismen als der wesentlichen Ursache der Stickstoffanreicherung im Boden gelangte, kann hier nicht eingegangen werden. Es steht jedenfalls heute fest, dass der Hauptanteil hierbei Bakterien zuzuschreiben ist. Solcher stickstoffsammelnder Bakterien kennen wir zur Zeit eine ganze Reihe.

Je nach ihrer Lebensart unterscheiden wir zwei Hauptgruppen derselben.

Zur 1. Hauptgruppe zählen sämtliche freilebenden stickstoffbindenden Schizomyceten.

Zur 2. Hauptgruppe zählen die in Symbiose mit höheren Pflanzen lebenden Bakterien.

Die 1. Hauptgruppe unterteilen wir wieder je nach dem Sauerstoffbedürfnis ihrer Vertreter in:

- (a) aerob lebende Stickstofffixierer,
- (b) fakultativ anaerob lebende und
- (c) obligat anaerob lebende, zur Stickstoffassimilation befähigte Bakterien.<sup>1</sup>

<sup>1</sup> Anmerk. Der grundlegende Unterschied zwischen den Anaerobionten und Aerobionten besteht nicht, wie man früher glaubte und auch heute vielfach irrigerweise noch annimmt—darauf ausdrücklich hinzuweisen, sei mir hier deshalb gestattet—darin, dass die Anaerobionten nur in einem absolut Sauerstofffreien Raum resp. Medium leben können, während die Aerobionten den normalen Sauerstoffgehalt der Luft unbedingt zur Atmung benötigen, sondern der Unterschied liegt, wie zuerst Arth. Meyer zeigen konnte, darin, dass die aerob lebenden Bakterien eine untere Grenze im Sauerstoffgehalt der Atmosphäre haben, unterhalb derer sie nicht mehr imstande sind zu leben, während es eine solche untere Grenze für die Anaerobionten nicht gibt. Wir kennen kein obligat

Als aerob lebende Stickstofffixierer wären hier die Vertreter der Azotobakter-Gruppe zu nennen. Als Repräsentant der zweiten Untergruppe gilt *Bac. asterosperus* und in die dritte Untergruppe sind alle Stickstofffixierenden *Clostridien* zu stellen, die wahrscheinlich, soweit sie nicht mit *Bac. amylobacter* A.M. et Bredem. völlig übereinstimmen, von diesem nur wenig differieren.

In die zweite Hauptgruppe gehören die Leguminosenknöllchenbakterien mit ihren zahlreichen Arten hinein.

### DIE AZOTOBAKTER-GRUPPE

Die Azotobakter-Gruppe umfasst zwei gut charakterisierte Species, und zwar *Azot. chroococcum* und *Azot. agile*, Arten, die sich durch einen grossen Formenreichtum auszeichnen und wohl deshalb Löhnis die Veranlassung gegeben haben zu den "Studien über den Lebenszyklus der Bakterien," bei denen er und sein Mitarbeiter (48) Ergebnisse erhalten haben, die noch recht umstritten sind. *Azot. Beij.* J. G. Lipman, *Azot. Smyrni* C. B. Lipman, *Azot. Vinelandii* Lipman und *Azot. vitreum* Löhnis sind keine neue Arten, sondern teils der einen teils der andern der obengenannten zwei Species zugehörig. Ihre gewöhnliche Form ist die ovale bis kugelige. Eigenbewegung ist bei beiden vorhanden. Durch ihr unterschiedliches Verhalten in der Bildung von Farbstoffen sind sie leicht zu erkennen und zu trennen. Im Hinblick auf ihre grosse Verbreitung hat Vageler (68) mit Recht die Azotobakterarten als echte Kosmopoliten bezeichnet, und B. Heinze (31) kommt auf Grund seiner Untersuchungen zu dem Schluss, dass es "azotobakterfreie Böden überhaupt nicht gibt." Es scheint aber, dass diese Bakteriengruppe in fast allen Kulturböden fehlt oder sicher nicht zur Wirkung gelangt, deren Reaktion unterhalb von pH 5,6 liegt, weil eine sauerere Beschaffenheit von Azotobakter nicht mehr oder selten vertragen wird (63). Das bestätigen auch die Versuche von Gainay (19), Gainay und Batcheler (20), Waksman (69), Johnson und Lipman (38), Yamagata und Itano (79), Gillespie (23), Petersen (57) u.a.; Niemeyer (54) fand als Grenzwert pH 5,0 und Niklas, Poschenrieder und Hock (55) geben an, noch bei pH 4,5—5 Azotobakter in den Böden gefunden zu haben. Diese Werte sind aber in kaliumchloridhaltiger Bodenlösung festgestellt worden. Die Organismengruppe ist überhaupt sehr empfindlich gegen Reaktionsänderungen; ihr Wachstumsoptimum bewegt sich nach meinen Untersuchungen in verhältnismässig engen Grenzen,

anaerobes Bakterium, das nicht bei einem geringen Sauerstoffgehalt leben könnte. Wir wissen andererseits heute sicher, dass die obligat anaeroben Bakterien auch bei normalem Sauerstoffgehalt der Luft sich entwickeln können, aber zum Unterschied von den fakultativ anaeroben, nur dann, wenn die Aerobionten den Anaerobionten erst die Entwicklungsmöglichkeiten schaffen, was nach Rippel "vielleicht auf dem Unschädlichmachen gewisser den Anaeroben schädlicher Stoffwechselprodukte seitens der Aeroben" und nicht auf dem Verbrauch des freien Sauerstoffs durch die letzteren beruht.

nämlich etwa zwischen pH 6,5 und 7,7,—ähnliches fanden Yamagata und Itano (79)—dagegen soll die stickstoffbindende Kraft nach Johnson und Lipman (38) in weiteren Grenzen gleichbleibend sein und zwar zwischen pH 6,2 und 8,8. Von mir (62) ausgeführte zellanatomische und mikrochemische Untersuchungen haben gezeigt, dass—entgegen den Angaben früherer Untersucher—Glykogen von Azotobakter als Reservestoff *nicht* gespeichert wird und die Hauptmenge der in den Zellen wirklich abgelagerten Reserveassimilate aus fettartigen Stoffen besteht, daneben aber auch noch eine Nukleinsubstanz, das Volutin, ständig vorkommt. Der Gehalt an letzterem Stoff ist abhängig von der Menge der zur Verfügung stehenden assimilierbaren Phosphorsäureverbindungen. Hinsichtlich der Verwendung von Kohlenstoffverbindungen als Energiequellen ist Azotobakter durchaus nicht sehr wählerisch, wenn es auch zahlenmässig weniger auszunutzen vermag, als eine Reihe anderer Bodenbakterien; andererseits kann nicht bestritten werden, wie ich gemeinsam mit Ruschmann (63) beweisen konnte, dass gerade dem Azotobakter ein feines Unterscheidungsvermögen für die Konfiguration des Moleküls eigen ist. Es ist bekannt, dass Azotobakter auch eine ganze Reihe von Stickstoffverbindungen verwerten kann, und es konnte von uns gezeigt werden, dass bei gleichzeitiger Darreichung von assimilierbaren Nitrat- und Ammonverbindungen ein sehr interessantes elektives Verhalten zutage trat, indem nämlich zuerst der Ammon- und dann erst der Nitrat-Stickstoff angegriffen wurde. Quantitative Untersuchungen ergaben, dass im Laboratoriumsversuch bei Darreichung von Nitrat bei Zimmertemperatur, unabhängig von der vorhandenen Energiequelle, nur 0,1–0,187 mg. Stickstoff pro Tag verbraucht wurden. Im Mineralsalzverbrauch ist Azotobakter äusserst ökonomisch, vermag aber andererseits noch relativ hohe Salzgaben ohne ernste Schädigung zu vertragen. Auch gegen Trockenheit ist Azotobakter wenig empfindlich.

### *Bacillus Asterosporus*

Der Repräsentant der fakultativ anaerob lebenden Stickstofffixierenden Bakterien, der *Bac. asterosporus*, ist ebenfalls weit verbreitet, und Brede-  
mann (11) konnte ihn aus vielen Erdproben der verschiedensten Weltge-  
genden gewinnen. Allerdings scheint er im Gegensatz zu Azotobakter  
mehr an den Kulturboden gebunden zu sein. Er gehört zu den Sporen-  
bildner und ist infolgedessen noch resistenter als Azotobakter. Seine  
relativ grossen, tonnenförmigen Sporen sind bei guter mikroskopischer  
Beobachtung leicht zu erkennen; die Exine derselben ist mit 8–12 feinen,  
ziemlich regelmässig angeordneten Längsleisten besetzt, welche ihrer-  
seits kleine Zäckchen tragen. Die peritrich begeisselten, gleichmässig  
gebauten Oidien (vegetative Stäbchen) schwellen vor der Sporangienbild-  
ung meist endständig an. Der Entwicklungszyklus von Spore zu Spore  
wickelt sich bei 28° C in etwa 54 Stunden vollkommen ab. An Reser-

vestoffen ist in den Stäbchen und vor allem den unreifen Sporangien reichlich Glykogen und auch Volutin festzustellen, nicht aber Fett. Ebenso wird niemals Iogen gebildet, zum Unterschied von *Bac. amylobacter*, der reichlich Iogen zu speichern befähigt ist und mit *Bac. asterosporus* bekanntlich morphologisch und auch im physiologischen Verhalten viel Ähnlichkeit hat. Zahlreiche Kohlehydrate und auch andere Kohlenstoffhaltige Energiequellen werden unter Gas- und Säurebildung durch *Bac. asterosporus* zersetzt. Besonderes Interesse erweckt die ungemein grosse Latitüde der Sauerstoffspannung, die für alle Morphoden, also sowohl Sporen wie Stäbchen und Sporangien zwischen 0 und 5500 mg. Sauerstoff im Liter liegt und die so gross bislang für eine andere Species wie *Bac. asterosporus* noch nicht bekannt ist. Auch gegen höhere Temperaturen ist er verhältnismässig widerstandsfähig. Die Abtötungszeit für die Sporen bei 100° C. liegt zwischen 7 und 13 Minuten, bei 80° zwischen 4 und 4½ Stunden.

### *Bacillus Amylobacter*

Bekanntlich verdanken wir Winogradsky unsere ersten Kenntnisse über das Vorkommen von anaeroben Stickstoffsammlern; durch seine ausgezeichneten Untersuchungen (74) über den von ihm *Clostridium Pasteurianum* benannten Anaerobionten erhielten wir Aufschluss über die Natur dieser Organismengruppe, zu der u.a. *Clostridium americanum* Pringsheim, *Clostridium α* und *Clostridium β* von Haselhoff und Bredemann, *Bac. amylobacter* von Gruber, *Bac. saccharobutyricus* von Klecki, *Granulobacter pectinovorum* Beij. sowie die Buttersäurebazillen von Freudenreich und von Jensen gehören, die Bredemann (12) unter dem Sammelnamen *Bac. amylobacter* A.M. et Bredemann zusammengefasst und ausführlich beschrieben hat. Winogradsky, J. Behrens (4), Benecke und Keutner (8), Haselhoff (28), H. Pringsheim (58) und Bredemann betonen sämtlich ihre starke Verbreitung. Die Sporen des *Bac. amylobacter* ähneln in der Form denen von *Bac. asterosporus*. Ihre Exine ist nicht immer ganz glatt, sondern lässt manchesmal auch kleine Zäckchen erkennen, zeigt jedoch niemals die Leistenverdickungen wie sie für *Bac. asterosporus* charakteristisch und bereits erwähnt sind. Eine Besonderheit von *Bac. amylobacter* ist aber die beim Sporenreifungsprocess um die Spore erhalten bleibende "Sporenkapsel", wie sie Winogradsky nennt.

Von Reservestoffen ist bei *Bac. amylobacter* nur Glykogen und Iogen nachweisbar und zwar ist die Speicherung dieser beiden Assimilate in bestimmten Entwicklungsstadien eine auffallend starke. Volutin ist bisher in keinem Lebensalter des Bakteriums gefunden worden, und auch Fett kommt normalerweise in den Zellen nicht vor. In der Gas- und Säureproduktion ähnelt *Bac. amylobacter* dem *Bac. asterosporus*. Er gedeiht besser auf festen Nährsubstraten wie in flüssigen, auch bei Kreidezusatz zu beiden. Hinsichtlich der ihm gebotenen Nährstoffe ist er weder anspruchsvoll noch wählerisch. Er vermag sich in absolut Stickstoff-

freien Nährlösungen zu entwickeln, verliert diese Fähigkeit jedoch sehr leicht, was Rippel (60) neuerdings auf das Fehlen in sehr geringer Menge wirksamer, noch nicht näher bekannter Substanzen in den künstlichen Substraten zurückführt. Von Stickstoffverbindungen bevorzugt er wahrscheinlich die anorganischen.

Die Resistenz gegen höhere Temperaturen ist etwas geringer als bei *Bac. asterosporus*. Die Tötungszeit bei 100° C. liegt zwischen 2 und 5 Minuten.

### DIE LEGUMINOSENKNÖLLCHENBAKTERIEN

Die Knöllchenbakterien der Leguminosen sind nicht nur in ihrer Sonderleistung als Stickstofffixierer, sondern ganz allgemein, eine der interessantesten Organismengruppen, die wir in der Bakteriologie kennen. Gemeinsam mit A. Müller habe ich (53) im vergangenen Jahre eine ausführliche Arbeit über die Biologie derselben mit besonderer Berücksichtigung ihrer Artverschiedenheit veröffentlicht, in der ein grosser Teil der vorhandenen, recht umfangreichen Literatur verwertet worden ist. Ich möchte deshalb hier nur kurz auf diese Bakteriengruppe eingehen und darauf hinweisen, dass es uns gelungen ist, konstante physiologische und kulturelle Verschiedenheiten festzustellen, auf Grund deren eine Erkennung und Diagnostizierung der einzelnen Arten resp. Untergruppen unter Ausschaltung des Pflanzenversuchs oder der serologischen Prüfung möglich ist. In einer im gleichen Jahre wie die unserige abgeschlossenen Arbeit hat W. H. Wright (76) über Verschiedenheiten berichtet, die sogar unter den einzelnen Bakterienstämmen derselben Pflanzenart, nämlich von Soja, vorhanden sind und sich mehrere Jahre hindurch erhalten haben. Auf Grund dieser Unterschiede kommt er zu einer Trennung der Sojabakterien in zwei Typen. Ob wir noch mehr Typen in den einzelnen Untergruppen haben, das werden erst die weiteren Forschungen erweisen müssen. Nach Ergebnissen von Wright und der vor ihm veröffentlichten von J. W. Stevens (64) und Bialosuknia und Klott (9) scheint es wieder zweifelhaft, ob die serologische Methode zur Klassifizierung der Bakterien in die einzelnen Untergruppen überhaupt herangezogen werden kann. Die Sojabakterien, die allein in einer Untergruppe stehen, können nach Leonard (43) auch an *Vigna sinensis* Knöllchen hervorrufen, ebenso, wie es ja bekannt ist, dass z.B. die serologisch von den Pisumbakterien unterscheidbaren Bakterien von *Vicia Faba* bei Pisumpflanzen Knöllchen erzeugen können und umgekehrt. Auch sollen nach Whiting und Hansen (72) die Bakterien von *Phaseolus lunatus* sich in Impfversuchen als identisch mit den Bakterien der *Vigna sinensis*-Gruppe erwiesen haben, aber verschieden sein von den *Phaseolus vulgaris*-Bakterien.

Unsere zellanatomischen Untersuchungen haben ergeben, dass die Knöllchenbakterien einen fettartigen Inhaltstoff und Volutin in sich ablagern, dass dagegen Glykogen und auch Iogen nicht gespeichert wer-



den. Die formative Reizwirkung verschiedener Stoffe wurde verfolgt und aus unseren Ergebnissen glauben wir uns zu dem Schluss berechtigt, dass die innerhalb der Knöllchen anzutreffenden, von der normalen Stäbchenform abweichenden Bakterien, die unkorrekter Weise noch heute den Namen "Bakteroiden" führen, nur als teratologische Gebilde und nicht als höhere Entwicklungsformen zu betrachten sind und der Einwirkung gewisser Reizstoffe oder osmotischen Änderungen zugeschrieben werden müssen. Aus diesem Grunde scheint es uns durchaus zweifelhaft, dass, wie scheinbar zumeist angenommen wird, nur diesen sogenannten Bakteroiden die Eigenschaft, Luftstickstoff zu binden, zukommen soll.

Über das Vermögen der Knöllchenbakterien, bei ganz verschiedenem pH zu wachsen und auch noch Knöllchen zu bilden, liegen eine Reihe von Untersuchungen vor. So hat O. C. Bryan (14) für 21 Sojabakterien-Stämme den kritischen pH-Wert bei 4,0–4,7 liegend gefunden und konnte Knöllchenbildung zwischen pH 4,6 und 8 erzielen.

### DIE STICKSTOFFBINDENDE KRAFT DER BAKTERIEN

Die stickstoffbindende Kraft der verschiedenen Organismen ist weitgehend abhängig von den Umweltbedingungen. Nötig ist bekanntlich in erster Linie das Vorhandensein einer verwertbaren Kohlenstoffquelle zur Lieferung der bei dem Stickstoffbindungsprozess erforderlichen Energie. Auf die Bedeutung der Bodenreaktion ist bereits hingewiesen worden. Über den Wert der Phosphorsäure haben uns u.a. die Untersuchungen von J. Stoklasa und Kříčka (65) und von S. A. Waksman und Karunakar (70) Aufschluss gegeben.

Die Ansicht, die A. Koch und Seydel (40) als die allgemein geltende hinstellen, dass nämlich die stickstoffbindenden Bakterien den von ihnen assimilierten freien Stickstoff nur zum Aufbau ihres Zellplasmas und nicht etwa auch zur Bildung eines Reservestoffs verwenden, trifft in so allgemeiner Form nicht zu, denn es wurde oben bereits erwähnt, dass z.B. *Azotobakter*, *Bac. asterosporus* und auch die Knöllchenbakterien Volutin, eine Nukleinsäure, also einen stickstoffhaltigen Stoff speichern, der doch jedenfalls sich wie das Zelleiweiss aus dem assimilierten Stickstoff aufbaut, worauf auch die Untersuchungsergebnisse von Fl. Mockeridge (52) hindeuten. Die von Koch aus dieser allgemeinen Ansicht gezogene Folgerung, die Stickstoffbindung höre mit der Zellvermehrung in einer Kultur auf, während der Verbrauch des Energiematerials noch lange Zeit weiter gehen könne, wurde scheinbar experimentell gestützt. In der Tat ging nach Koch und Seydel die stärkste Stickstoffbindung im Laboratoriums-Versuch durch *Azotobakter* innerhalb der ersten 5–8 Tage vor sich, liess dann aber nach, während der Verbrauch der Energiequelle ungleich stärker anhielt, sodass nach längerer Kulturdauer sich das Verhältnis des gebundenen Stickstoffs zu verbrauchter Energiequelle wesentlich verschob. Da der Stickstoffhaltige Reservestoff, das Volutin,—genügende Mengen

Phosphorsäure zum Aufbau derselben vorausgesetzt—bereits in relativ jungen Zellen gespeichert und später nicht mehr sichtlich vermehrt wird, so behalten die Untersuchungsbefunde von Koch und Seydel *ihren vollen Wert*; es dürfte der Stillstand in der Zellteilung allerdings nicht genau mit dem Stillstand in der Stickstofffixierung zusammenfallen. Dass der Energieverbrauch nicht allein von der Zellvermehrung in der Kultur abhängig ist, ist auch später von E. R. Allen (1) bestätigt worden. Es ergeben daher alle diejenigen Versuche, bei denen die Ermittlung des Stickstoffgewinnes und des Energieverbrauchs *erst nach längerer Zeitdauer* vorgenommen worden ist, und scheinbar ist das bei den meisten früheren Untersuchungen der Fall, kein klares Bild. Eine gewisse Bestätigung der Befunde von Koch und Seydel scheinen die Versuche von Hoffmann und Hammer (35) zu erbringen, die, obwohl sie nicht mit Reinkulturen ausgeführt sind, hier von Interesse sein dürften. Es wurden von ihnen bei Darreichung von 0,5 Prozent Mannit 11,4 mg. Stickstoff pro g. Mannit, bei Gaben von 1 Prozent nur 8,25 mg. und zwar nach 28-tägiger Bebrütungsdauer gefunden. In beiden Fällen war das Mannit nach dieser Zeit restlos aufgebraucht. Im zweiten Fall konnte also der Kraftverbrauch infolge der grossen Mannitgabe fortschreiten, nachdem die Stickstoffbindung im wesentlichen beendet war, im ersten Fall war die Energiequelle bereits viel früher verbraucht. Wenn Hoffmann und Hammer aus ihren Befunden schliessen, dass bei Darreichung geringerer Gaben von Zucker eine stärkere Stickstoffbindung stattfindet, so ist das damit also durchaus nicht bewiesen und bei so geringen Mengenunterschieden nicht einmal wahrscheinlich. Die Befunde Hunters (36) bei 4 Tage alten Kulturlösungen mit 0,5 und 1 Prozent Dextrose sprechen sogar dagegen. Die Ergebnisse von Gerlach und Vogel (22), die bei Versuchen mit 0,1–1,5 Prozent Dextrose bei 1,2 Prozent die stärkste Gesamtausbeute, nämlich 127,9 mg. Stickstoff auf 1 Liter Kulturflüssigkeit (12 g. Dextrose), festgestellt hatten, sind für die Entscheidung dieser Frage hier nicht verwertbar, weil die quantitativen Bestimmungen erst nach fünf wöchiger Kulturdauer zur Durchführung kamen. Die Untersuchungsergebnisse von Ashby (2) mit Azotobakter aus verschiedenen Böden erheischen ebenfalls eine andere Auswertung als sie erhalten haben, denn es wird jetzt verständlich, dass Azotobakter aus Rothamsted-Erde nach vierzig-tägiger Kulturdauer weniger (nur 4,62 resp. 4,71 mg.) Stickstoff pro Energieeinheit gebunden hatte als Azotobakter z.B. aus Kairo-Erde nach zwanzig-tägiger Kulturdauer (5,73 resp. 7,64 mg.).

Hunter (37) fand bei Versuchen mit durchlüfteten Azotobakter-Kulturlösungen im Jahre 1923 innerhalb der ersten 4 Tage 64 Prozent der dargebotenen Dextrosemenge verbraucht und 65 Prozent der am Ende (8. Tage) festgestellten Gesamt-Stickstoff-Menge gebunden. Daraus zieht er den Schluss, dass eine sehr enge Beziehung zwischen Energieverbrauch und Stickstoffbindung besteht. Das ist sicherlich für Zeiträume

bis zu 8 Tagen zutreffend und mit den Befunden von Koch und Seydel—die Hunter scheinbar unbekannt waren—gut in Einklang zu bringen, dagegen ist auch hier eine Verallgemeinerung, nach der eine solche Korrelation stets besteht, nicht statthaft, denn die Resultate von Koch und Seydel zeigen ja, dass das Fortschreiten des Energieverbrauchs in älteren Kulturen unabhängig von Zellvermehrung und Stickstoffbindung ist. Die Stickstoffgewinne, die Hunter erhielt, blieben weit hinter denen von Koch und Seydel zurück; im Höchstfall wurden 15,9 mg. Stickstoff pro g. verbrauchter Dextrose festgestellt. Ob das allein an der Verschiedenartigkeit der Substrate liegt, kann nur experimentell entschieden werden. Auffallend ist allerdings, dass so hohe Gewinne, wie sie Koch und Seydel auf Dextroseagar erzielt haben, nämlich 80,6 mg. Stickstoff pro g. Energiematerial, noch nicht wieder gefunden worden sind. Auch Winogradsky (75) hat bei seinen neuesten Erd-Untersuchungen nur durchschnittlich 10 mg. Stickstoff auf 1 g. Energiestoff erhalten.

Dass Stoklasa (66) bei seinen Respirationsversuchen mit *Azotobakter* die höchste Kohlendioxydproduktion bis zum 10. Tage in der Kultur fand, deckt sich ebenfalls mit den von Koch und Seydel gemachten Beobachtungen, dass in diesen Tagen Zellvermehrung und Stickstoffassimilation am stärksten sind und infolgedessen auch die stärkste Arbeitsleistung in diese Zeit fällt.

C. B. Lipman und L. Teakle (46) konnten zeigen, dass mit *Azotobakter* selbst in Erde, deren lösliche Kohlenstoffverbindungen zuvor ausgezogen worden waren, noch ganz ansehnliche Stickstoffgewinne zu erzielen sind, nämlich 1 mg. Stickstoff auf etwa 70 mg. der Gesamtmenge an Kohlenstoff, von dem aber jedenfalls nur ein Teil in einer für *Azotobakter* verwertbaren Form vorhanden war.

Für *Bac. amylobacter* fand Winogradsky in Reinkultur pro g. verbrauchten Zuckers nur etwa 1,5–1,8 mg. Stickstoff, bei seinen bereits erwähnten neuen Untersuchungen mit frischer Erde durchschnittlich 5 mg. Mit Zellulose, die von *Bac. amylobacter* allein nicht ausgenutzt werden kann, wurden von Pringsheim (58) Stickstoffgewinne bis zu 10,4 mg. erzielt, wenn er gleichzeitig zelluloselösende Bakterien der Kulturlösung zusetzte. Da auch bei diesen letzteren Untersuchungen die quantitativen Stickstoff- und Kohlenhydrat-Bestimmungen erst nach längerer Kulturdauer vorgenommen worden sind, haben sie ebenfalls nur bedingten Wert. Interessant sind in diesem Zusammenhang noch einige Analysenergebnisse Bredemanns mit *Bac. amylobacter*. In einem Versuch (12) (Kolben 7, 8, 9, Tabelle II p. 506), bei dem die Kulturlösungen, zum Unterschied von den übrigen, keinen Kreidezusatz erhielten, schwankte der Verbrauch an Dextrose—die Untersuchungen wurden nach 4–6 Monaten vorgenommen—zwischen 1,87 und 2,12 g. (sonst zwischen 7,5 und 20 g.) und die Stickstoffgewinne betrugen 5,61–6,67 mg. Stickstoff pro g. Zucker (sonst nur bis 3 mg.!). Der geringe Zuckerverbrauch und die dadurch bedingte

höhere Stickstoffausbeute dürfte ihre Ursache vielleicht darin haben, dass infolge der eigenen Säurebildung die Bakterien selbst derart geschädigt wurden, dass sie nach einer kurzen Entwicklung—und Stickstoffbindungszeit die restliche noch unzersetzt vorhandene Zuckermenge nicht mehr zur Lebensenergie verwenden konnten und so nach mehrmonatiger Untersuchung selbstverständlich höhere Gewinne pro Energieeinheit festgestellt werden mussten wie in allen denjenigen Fällen, wo der Kreidezusatz die die Lebensenergie schädigenden freien Säuren beseitigt hatte.

Über die stickstoffbindende Kraft von *Bac. asterosporus* liegen, soweit mir bekannt, nur Untersuchungen von Bredemann aus dem Jahre 1909 (11) vor, die zwar zeigen, dass innerhalb von 6 Monaten Gesamtstickstoffgewinne bis zu 60 mg. erzielt wurden, das ist umgerechnet auf die in dieser Zeit verbrauchte Zuckermenge 3,00 mg. pro Energieeinheit, die aber, da ebenfalls erst nach so langer Kulturdauer analysiert, keine Beziehung erkennen lassen zwischen der stickstoffbindenden Kraft und dem Zuckerverbrauch im Sinne von Koch.

*Es scheint also wichtig, an dieser Stelle darauf hinzuweisen, dass bei ferneren Untersuchungen in dieser Richtung die Koch'schen Argumente Berücksichtigung finden.* Man wird dann wahrscheinlich bei Verwendung von Reinkulturen in vielen Fällen zu bedeutend günstigeren Ergebnissen kommen.

Bei den in Symbiose mit höheren Pflanzen lebenden stickstoffbindenden Bakterien haben sich experimentell noch keine brauchbaren Prüfungen durchführen lassen. Die Züchtung dieser Bakterien in stickstofffreien Medien können uns naturgemäss kein Bild davon geben, wie gross die stickstoffbindende Kraft innerhalb des Pflanzenkörpers ist. Ob die Knöllchenbakterien ausserhalb der pflanzlichen Gewebe überhaupt Stickstoff assimilieren können, ist noch strittig; nach Untersuchungen von Mazé (51), Löhnis (47), Lewis und Nicholson (44), Golding (24), Greigh-Smith (27), Fred (18), Hills (32), und Singh (61) ist es der Fall. Auch Beijerinck (5) hat das zuerst bestätigt, später (6) aber widerrufen. Nach den neuesten Untersuchungen von Barthel (3) findet eine Stickstofffixierung durch Knöllchenbakterien ausserhalb der Pflanze *nicht* statt. Beijerinck (6) bestreitet sogar, dass die Knöllchenbakterien überhaupt zur Stickstoffbindung befähigt sind. Dass innerhalb der Knöllchen keine Stickstoffassimilation stattfindet, hat er auf gasometrischem Wege zu beweisen versucht. Er ging so vor, dass er Knöllchen von Lupinen und Serradella in Mengen von 100, 500 und 1000 g. in weite Glasflaschen brachte, die er mit der Gasbürette verband und bei 25° C aufstellte. Nach 12–20 Tagen wurde nach Ableiten des Kohlendioxyds und Sauerstoffs das Stickstoff Gas bestimmt. In einigen Versuchen wurden auch "die Wurzeln mit den Knöllchen und mit grossen Stücken vom Stengel vereint", in gleicher Weise gasometrisch geprüft. Alle diese Versuche

fielen aber negativ aus. Dem Einwurf, dass derartige Untersuchungen mit ganzen unversehrten Pflanzen durchgeführt werden müssten, glaubt Beijerinck dadurch begegnen zu müssen, dass er es für höchst unwahrscheinlich erklärt, dass die Stickstoffbindung mit dem Wachstum der Knöllchen verbunden sein solle. Er lässt aber völlig ausser acht, dass die Stickstoffbindung innerhalb der Knöllchen sicherlich sehr weit von der Verdunstungsgrösse der Pflanzen abhängig sein wird und demnach die Transpiration ein äusserst wichtiger Faktor ist; es genügt keineswegs, die Knöllchen allein oder mit Pflanzenteilen einfach der Luft auszusetzen, da damit kein normaler Gasaustausch innerhalb der Knöllchen gewährleistet ist. Auf den hohen Stickstoffgehalt der Knöllchen, der bis zu 6 prozent betragen kann, entsprechend einem Eiweissgehalt von fast 40 prozent, macht er selbst aufmerksam, ohne aber eine Erklärung dafür zu finden. Es dürfte also die bisher geltende Anschauung, dass den Knöllchenbakterien die Fähigkeit der direkten Stickstoffbindung innerhalb der Leguminosenknöllchen zukommt, damit nicht erschüttert sein.

#### DER CHEMISMUS DER STICKSTOFFBINDUNG

Auf die Frage, wie ist es diesen kleinsten Lebewesen nur möglich, einen durch so grosse Indifferenz ausgezeichneten Stoff wie den gasförmigen Stickstoff zu assimilieren, ihn also zu einer chemischen Bindung zu zwingen, muss auch heute noch die Antwort lauten: ignoramus. Wir wissen nur, dass die zur Bindung des atmosphärischen Stickstoffs befähigten freilebenden Organismen den assimilierten Stickstoff vollständig oder zum grössten Teil zum Aufbau von Körpersubstanz, teilweise auch nukleinsäuren Reservestoffen verwenden und dass dementsprechend die Endprodukte stickstoffhaltige hochmolekulare organische Verbindungen sein müssen. Betreffs der auf dem Wege dieser Stickstoffassimilation entstehenden Anfangs- und Zwischenprodukte sind unsere Kenntnisse noch durchaus unvollkommene.

Dass auch bei den Knöllchenbakterien die Endprodukte hochmolekulare organische Stickstoffverbindungen sein werden, wird ziemlich allgemein angenommen. Hiltner (34) war allerdings der Meinung, dass vielleicht ein aus den Bakterienzellen austretendes "Kernplasma" bei der Vereinigung mit von der Leguminosenpflanze herrührenden Stoffen unbekannter Natur innerhalb der Knöllchen (aber ausserhalb der Bakterienzelle) die Stickstoffbindung bewirke. Ich habe mich sehr eingehend mit der Anatomie und Chemie der Knöllchenbakterienzellen beschäftigt und in meinen Beobachtungen *niemals* solche Aussprossungen von "Kernplasma" beobachtet. Es scheint mir deshalb ziemlich sicher und die Abbildung, die Hiltner im Lafar (Bd. 3. 1904/06. p. 52. Fig. 9) von Sojabakterien mit "Kernplasma" gab, bestärken mich darin, dass es sich um Bilder handelt, die nicht die natürlichen Verhältnisse bei den Bakterienzellen wiedergeben, sondern entstanden sind vielleicht durch Verwendung

von zu konzentrierten Farblösungen, vielleicht durch zu starkes Antrocknen oder auch uz warmes Färben, bei dem also jedenfalls ungleichmässige *Schrumpfungen* der reservestoffhaltigen Zellen eingetreten sind, die solche Aussprossungen vorgetäuscht haben. Die von Beijerinck und van Del-den (7) 1902 aufgestellte Behauptung, dass bei der Assimilation des freien Stickstoffs zunächst eine lösliche Stickstoffverbindung unbekannter Art entstünde, die von dem aktiven Organismus ausgeschieden würde, war auf irrige Voraussetzungen aufgebaut und ist später berichtigt worden [siehe Alfr. Koch im Lafar. (Bd. 3. p. 7)]. Nach Untersuchungen von Whiting und Schoonover (73) an *Vigna signensis*, war in den verschiedensten Altersstufen der pflanzen und ihren Knöllchen weder Ammoniak noch salpetrige oder Salpeter-Säure nachweisbar, woraus der Schluss gezogen wird, dass die Stickstoffbindungsreaktion eher organischer als anorganischer Natur sein werde.<sup>1</sup>

Winogradsky (74) bezeichnete es, nachdem er bei *Clostridium Pasteurianum* die Bildung von Wasserstoff-Gas beobachtet hatte, als wahrscheinlich, dass sich der Wasserstoff in statu nascendi bei diesen Mikroben innerhalb des Zellleibes mit dem atmosphärischen Stickstoff zuerst zu Ammoniak verbinde. Reinke (59) war der Ansicht, dass bei allen stickstoffbindenden Organismen auf diesem Wege zuerst Ammoniak entstehe und hob hervor, dass so der Stickstoff gleich an Wasserstoff als demjenigen Element gebunden sei, an dem er auch im Eiweissmolekül vorkomme. Die neueren Untersuchungen von Warburg und Naegelein (71), die zeigen dass in der Pflanzenzelle Salpetersäure zu Ammoniak reduziert wird und also nur aus diesem Eiweiss aufgebaut werden kann, sprechen scheinbar für diese Annahme. Es ist aber zu berücksichtigen, dass nur die Amylobakter-Gruppe und *Bac. asterosporus* von den bekannten Stickstofffixierern gasförmigen Wasserstoff bilden; hier ist also eine derartige Stickstoffbindung durchaus möglich, dagegen hielt man bisher ganz allgemein eine solche Art der Stickstoffassimilation bei den übrigen, nicht gasbildenden Bakterien für unwahrscheinlich.

Da auf rein chemischem Wege durch direkte Anlagerung von Stickstoff an organische Kohlenstoffverbindungen amidartige Körper erhalten werden können, vermuteten Gerlach und Vogel (22), Lipman (45) und Heinze (29), dass auch die Stickstoffbindung innerhalb der Bakterien auf diese Weise vor sich gehe und aus den einfachen Amiden schliesslich hochmolekulare stickstoffhaltige Körper entstehen. Die Meinung, dass es sich bei der Stickstoffbindung um einen biologischen Oxydationsprozess handele, dessen Produkte salpetrige Säure resp. Salpetersäure seien, wird von Gautier und Drouin (21), Bonazzi (10) und Greaves (26) vertreten.

<sup>1</sup> Es wurde von Whiting und Schoonover auch die interessante Feststellung gemacht, dass in geimpften Pflanzen von *Vigna signensis* (cowpea) 9 Tage nach dem Aussäen in Stickstofffreien Sand die erste Stickstoffbindung nachweisbar wird und der Stickstoffgehalt nach 26 Tagen bereits 3mal so gross ist, als derjenige der ausgelegten Samen.

Loew (49) glaubte aus der Tatsache, dass feuchtes Platinmohr bei Berührung mit Luft und in Gegenwart von Basen Spuren von Ammonnitrit bildet, schliessen zu können, dass die Bakterien wohl in ähnlicher Weise den Stickstoff assimilieren. "Bestände eine solche Analogie wirklich," so meint Alfr. Koch (39), "dann könnte die moderne Anschauung von der Ähnlichkeit der Eigenschaften der Metallsole mit denen der Enzyme zu Versuchen führen, stickstoffbindende Enzyme aus Bakterien zu isolieren." Hiltner (34) hat anscheinend an eine baldige Verwirklichung dieser Idee geglaubt, seine in dieser Hinsicht so hoffnungsfreudig aufgenommenen Versuche mit Knöllchenbakterien haben aber zu einem positiven Ergebnis nicht geführt. Auch Herm. Fischer (17) sieht die stickstoffbindende Kraft als eine physiologische Leistung der Zelle an, die mit Hilfe von Enzymen ermöglicht werden soll. Diese Hypothese würde gestützt, so meint er, wenn es gelänge, "ebenso wie durch das Buchner'sche Verfahren zur Gewinnung der Hefenenzyme, auch aus Stickstoff-Bakterien Enzyme freizumachen und mit diesen die Luftstickstoffbindung durchzuführen."

Bis jetzt ist aber über die Existenz derartiger stickstoffbindender Enzyme nicht das geringste bekannt.

Stoklasa (65), der auch bei Azotobakter Wasserstoffbildung nachgewiesen haben will, was bestimmt nicht zutreffend ist, spricht diesem Wasserstoff in statu nascendi eine bedeutsame Rolle bei der Stickstoffbindung durch Azotobakter zu und vermutet, dass als erstes Produkt bei der Eiweissynthese Cyanwasserstoff entstehe, der auch tatsächlich in der Pflanzanzelle schon nachgewiesen sei. (In Sojaknöllchen hat W. H. Strowd (67) Cyanverbindungen nicht einmal in Spuren zum Nachweis bringen können.)

Wenn neueste Untersuchungen von Kostytschew, Ryskaltchouk (41) und Schezowa (42) bei Nachprüfung Bestätigung finden, so bildet auch Azotobakter als restes Produkt der Stickstofffixierung durch direkte Reduktion von molekularem Stickstoff Ammoniak, wozu nach den russischen Untersuchern ein sehr kräftig wirksames reduzierendes Ferment vorhanden sein muss, dessen Nachweis aber noch aussteht.

Dass Reservestoffe wie z.B. das Glykogen bei der Stickstoffassimilation von Azotobakter und den Knöllchenbakterien beteiligt sind, wie Heinze (30) das ausspricht, ohne allerdings eine Erklärung dafür zu geben, in welcher Weise und in welcher Form er sich die Beteiligung denkt, ist deshalb unmöglich, weil von uns nachgewiesen werden konnte, dass dieser Stoff entgegen den früheren Angaben in der Literatur überhaupt nicht in den genannten Bakterien gespeichert wird. Wenn Löhnis in seinem Handbuch der landwirtschaftlichen Bakteriologie von 1910 p. 655/56 von ihnen sich mit Jod rotbraun färbenden Zelleinschlüssen sagt: "Sie scheinen mit dem Stickstoffassimilationsprozess in engem Zusammenhang zu stehen," so dürfte dieser Ausspruch wohl nur auf die irrigen Untersuchungen

sergebnisse von Heinze und anderen über die Zellinhaltstoffe der Knöllchenbakterien zurückzuführen sein.

Völlige Klarheit über den Stickstoffbindungsprozess bei den verschiedenen Bakteriengruppen, der wohl jeden landwirtschaftlichen Bakteriologen ausserordentlich interessieren dürfte, wird uns erst die Zukunft bringen müssen.

### DER ENERGIEBEDARF DER BAKTERIEN FÜR DIE STICKSTOFFASSIMILATION

In engem Zusammenhang mit der Chemie der Stickstoffbindung steht die Frage nach der Grösse des Energiebedarfs bei der Stickstoffbindung durch die Bakterien.

Bei den freilebenden stickstoffbindenden Arten hat man vielleicht einen gewissen Anhalt zur Ermittlung ihres Energiebedarfs an dem Nahrungsverbrauch in stickstofffreien Kulturmedien. Die verbrauchte Menge von Kohlehydraten lässt—wenigstens innerhalb der ersten 8–10 Tage des Versuchs—einen Rückschluss zu auf die zur Stickstoffbindung benötigte Energie.

Christiansen-Weniger (15) hat derartige Berechnungen für Azotobakter angestellt. Wenn er den durchschnittlichen Energiegehalt der organischen Substanzen, wie sie den Bakterien in Form von Mannit, Kohlehydraten etc. zugeführt werden, gleich 4000 Kal. pro kg. annahm und für 1 g. verbrauchte Nahrung nach Benecke 10 mg. Stickstoffgewinn setzte, so errechnete er für das kg. gebundenen N's einen Kraftverbrauch von 464 Kilowattstunden. Das ist, wenn man zum Vergleich den Energiebedarf der Technik zur Stickstoffbindung heranzieht, ein *sehr* hoher, werden doch z.B. zur Gewinnung des NO aus N+O bei der Salpetersäurefabrikation zur Bindung von 1 kg. Stickstoff nach ihm nur etwa 68 Kilowattstunden verbraucht, trotzdem es sich hierbei um einen endotherm verlaufenden Prozess handelt; bei der exotherm vor sich gehenden Bildung von Kalkstickstoff werden nach Caro sogar nur insgesamt 47,5–49,5 Kilowattstunden pro 1 kg. gebundenen Stickstoff benötigt.

Es wäre also der Energieverbrauch bei Azotobakter etwa 10 mal so gross wie bei der Kalkstickstofffabrikation. Inbegriffen in diese Zahl ist dabei jedoch der Anteil, der zum Leben verbrauchten Energie und derjenige, der zur Überführung der einfachen Stickstoffverbindung in hochwertige Eiweissprodukte benötigt wird. Nach Abzug des ersteren Anteils, der sich aus der bei der Dissimilation entstehenden Kohlensäuremenge errechnen lässt—für die Grösse des Energiebedarfs des letzten Anteils, der auf die Eiweissynthese entfällt, haben wir leider keinen Anhaltspunkt—verbliebe zwar nur noch die Hälfte, nämlich 232 Kilowattstunden, es ist dies aber immer noch ein so gewaltiger Energieverbrauch, dass uns berechtigte Zweifel kommen müssen, ob im normalen Naturgeschehen eine solche Kraftvergeudung tatsächlich stattfindet.



Es ist durchaus möglich, dass im Erdboden unter günstigem Einfluss anderer Faktoren und unter Mitwirkung anderer Mikroben sich der Energieverbrauch vorteilhafter gestalten wird, wie wir ihn auf Grund von Laboratoriumsversuchen feststellen können.

Immerhin scheint es, dass bei den Azotobakterarten, und wahrscheinlich kann man das auch für die anderen freilebenden Stickstoffsammler annehmen, der Stickstoffbindungsprozess ein *endothermer* ist.

Den Energiebedarf der Knöllchenbakterien hat Christiansen-Weniger auf experimentellem Wege zu ermitteln versucht; er war sich der Schwierigkeiten dabei von vornherein wohl bewusst. Auf die mit guter Überlegung angestellten Versuche kann hier natürlich nicht näher eingegangen werden, es sei aber vorweg gesagt, dass es wünschenswert erscheint, derartige Versuche unter Benutzung anderer Leguminosen zu wiederholen und unter Anwendung anderer, wenn möglich, verfeinerter Methoden in ihrer Auswertung exakter zu gestalten.

Jedenfalls zeigte sich bereits bei den Voruntersuchungen, die mit *Vicia Faba* zur Durchführung kamen, dass die Vermutung, die Leguminosen vermöchten einen starken Energiebedarf der Knöllchenbakterien durch erhöhte Assimilation zu decken, nicht zutraf. Es ist bei erheblichen Ernten an gebundenem Luftstickstoff in keinem Falle trotz starker Beschränkung der Assimilationsmöglichkeit eine beachtenswerte Minderernte an Trockensubstanz festzustellen gewesen. Daraus wird gefolgert, dass der Energiebedarf der Knöllchenbakterien ein äusserst geringer sein muss.

Aus dem Ergebnis des Hauptversuchs, dessen Auswertung allerdings unsicher wird dadurch, dass sich bestimmte Faktoren nicht getrennt erfassen lassen, berechnet Christiansen-Weniger einen Energieverbrauch von 22,4–28,88 Kal. pro g. Stickstoff. Diese Energiemenge ist derart gering, dass sie nicht einmal dazu ausreichen würde, den Bedarf der Knöllchenbakterien an Lebensenergie zu decken. Es muss demnach der Prozess der Stickstoffbindung durch die Knöllchenbakterien ein *exothermer* sein, und es wird weiterhin wahrscheinlich, dass die bei diesem exothermen Assimilationsvorgang freiwerdende Energie von den Bakterien noch in Lebensenergie umgewandelt werden kann.

## WIRKSAMKEITSSTEIGERUNG UND IMPFUNG

Wie ein Bakterienstamm auf einem bestimmten Substrat besser wächst als ein anderer Stamm derselben Species, so hat sich auch herausgestellt, dass z.B. ein Knöllchenbakterienstamm sich wirksamer erweisen kann als ein anderer von der gleichen Pflanzenart. Wright (76) hat, wie erwähnt, gezeigt, dass sich unter seinen Sojabakterienstämmen 2 Typen feststellen liessen, die serologisch und physiologisch voneinander unterscheidbar waren. Diese Typen verhielten sich in ihrer Wirksamkeit nach ihm (77) auch deutlich verschieden. Nach Hiltner (33, 34) sind die

in Knöllchen von Nebenwurzeln lebenden Bakterien "virulenter" als die in Knöllchen von Hauptwurzeln derselben Leguminose befindlichen, denn er nimmt an, dass die Bakterien nur dann von aussen in die Nebenwurzel einzudringen vermöchten, wenn ihre "Virulenz" diejenige der Bakterien der Hauptwurzelknöllchen überträfe. Wenn diese Annahme richtig wäre, so müsste man also bei Impfungen mit Zerreibungen von Wurzelknöllchen der Nebenwurzeln bessere Erfolge erzielen, als wenn man die Knöllchen der Hauptwurzel der gleichen Pflanze in Zerreibung zum Impfen verwenden würde. Dasselbe würde dann auch jedenfalls zutreffen, wenn man Reinkulturen aus den Knöllchen dieser Wurzeln benutzte. In mehrjährigen Feldversuchen habe ich diese Frage zu klären versucht. Als Versuchspflanzen dienten mir *Soja hispida* (die braune und die schwarze Varietät) und *Lupinus mutabilis*. Als Impfmateriale dienten die wässerigen Aufschwemmungen von Knöllchenzerreibungen (1) der Hauptwurzel (2) der Nebenwurzel. Ferner Bakterienreinkulturen (a) der Hauptwurzelknöllchen (b) der Nebenwurzelknöllchen. In allen Versuchsserien hat das Ergebnis nicht die Bestätigung der Hypothese Hiltners gebracht; es war eine stärkere Wirksamkeit der Bakterien aus Nebenwurzelknöllchen gegenüber denen aus Hauptwurzelknöllchen nicht festzustellen. Auf Einzelheiten meiner Versuche kann ich hier nicht eingehen, sie werden an anderer Stelle veröffentlicht.

Nun haben neuerdings Ehrenberg (16) und sein Schüler Wunschik (78) über positive Erfolge hinsichtlich der Wirksamkeitssteigerung von Knöllchenbakterien berichtet, die sie durch mehrfache Pflanzenpassage bei Pelusken, Wicken, Serradella und gelben Lupinen erzielt haben, während bei Rot- und Weissklee derartige Wirksamkeitssteigerungen nicht erreicht wurden. Die Ergebnisse sind von so weittragender Bedeutung, dass ich eine baldige Nachprüfung für geboten hielt. Doch schien mir nötig, die wichtigen Feststellungen der Knöllchenzahl resp.—masse nicht durch die subjektiven Beeinflussungen zu grossen Spielraum lassende Methode der "Bonitierung", wie sie Wunschik angewandt hat, sondern auf genauere Art vorzunehmen. Aus den Vegetationsgefässen, in denen die Versuche zur Durchführung kommen, lässt sich die Erde sehr wohl mit Wasser derart herauschlämmen, dass die Zählung der Knöllchen aller Pflanzen sicher möglich ist. Als Versuchspflanzen wählte ich worläufig Pelusken (*Pisum arvense*) und Serradella (*Ornithopus sativus*). Ich vermag das gleichmässige Ansteigen der Knöllchenmasse durch mehrfache Pflanzenpassage z.B. bei *Pisum*, wie es aus Wunschiks entsprechender Tabelle (Tab. 1) ersichtlich ist, auf Grund meiner letztjährigen Versuche allerdings nicht zu bestätigen, habe auch nicht beobachten können, dass die Knöllchen in den Vegetationsgefässen, die mit Knöllcheninfus der 2. oder 3. Pflanzenpassage geimpft waren, durchschnittlich grösser gewesen wären als diejenigen, die durch Impfen mit Erde oder Knöllcheninfus der 1. Pflanzenpassage hervorgerufen worden waren.

Ich möchte aber die Versuche mit den Reinkulturen erst beenden, ehe ich endgültig zu diesem ausserordentlich wichtigen Problem Stellung nehme.

Ob auf diese oder eine andere Weise experimentell eine Wirksamkeitssteigerung der Leguminosenknöllchenbakterien erzielt werden kann, die sich auch für die *Praxis* auswerten lassen wird, darüber kann sicheres zur Zeit nicht gesagt werden und es wäre unbedingt verkehrt, sich durch apodiktische Urteile über die Aussichtslosigkeit solcher Forschungen von weiteren Untersuchungen in dieser Richtung abhalten zu lassen.

Im allgemeinen sind die Stickstoffgewinne die durch die übliche Impfung mit den entsprechenden Reinkulturen im Boden erzielt werden befriedigend,<sup>1</sup> sie sind aber, wie das z.B. die Untersuchungen von P. E. Brown und J. H. Stallings (13) für Klee und Luzerne ergeben haben, nicht bei allen Leguminosen gleich hoch, sondern wechselnd je nach der Pflanzenart, der Bodenbeschaffenheit und den allgemeinen Wachstumsbedingungen. Da die Unkosten für die zum Impfen notwendigen Bakterienkulturen unbedeutend sind, ist in jedem Falle—sofern nicht bereits im Vorjahre die gleiche Leguminosenart zum Anbau auf dem gleichen Feld gewählt wurde, was wohl selten der Fall sein dürfte—die Impfung anzuraten. Dabei scheint mir allerdings die Forderung nach meinen Erfahrungen zu weitgehend, dass man z.B. für blaue Lupinen Knöllchenbakterien von blauen Lupinen und für gelbe Lupinen solche aus gelben Lupinen zum Impfen verwenden soll. Auch die Untersuchungsergebnisse von A. T. Perkins (56) mit verschiedenen Sojavarietäten nehmen dieser Forderung ihre Berechtigung.

Durch die guten Erfahrungen der Bodenimpfung beim Anbau von Leguminosen ermuntert, ist man schon früh auf die Idee gekommen, den Boden auch mit freilebenden stickstoffbindenden Bakterien zu impfen. Die dabei mit Azotobakter gemachten Erfahrungen sind aber keineswegs ermutigend gewesen. Die negativen Ergebnisse können eigentlich auch nicht verwundern, muss doch bedacht werden, dass das Azotobakter an und für sich schon weit verbreitet und demnach jedenfalls in den Versuchsböden bereits zugegen gewesen ist. Aussicht auf Erfolg haben wir auch ohne Impfung, wenn es uns gelingt, im Boden Bedingungen zu schaffen, die den stickstoffbindenden Bakterien eine kräftigere Vermehrung und damit verbundene stärkere Stickstoffassimilation ermöglichen. Auf Böden allerdings, deren pH bei 6 und noch darunter liegt, würde Impfung mit Azotobakter vielleicht Erfolg haben, nachdem die Bodenreaktion in dem für diesen Stickstofffixierer günstigen Sinne korrigiert wäre. Nach vorläufigen Mitteilungen von Makrinoff (50) ist auch dann Erfolg zu verzeichnen, wenn eine Mischung von Azotobakter und Cellulose-zersetzenden Bakterien zum Impfen verwandt wird und die künstliche

<sup>1</sup> Vergl. z.B. Fred (Soil Sci. 11, 1921: 469) und Fred, Wright und Frazier (ebenda 479). Die letztere Arbeit zeigt, dass bei Erbsen selbst auf ungekalktem, saurem Leimboden Impfung Erfolg haben kann; ferner Makrinoff (Soil Sci. 17, 1924: 19).

Düngung entsprechend den Bedürfnissen der zu bauenden Pflanzenart und unter Berücksichtigung des physiologischen Verhaltens der Mikroorganismen gewählt wird.

Azotobakter-Impfungen würden ausserdem bestimmt von Bedeutung und Vorteil werden, wenn es gelänge, nicht ubiquitäre Species mit überragendem Stickstoffbindungsvermögen zu finden oder es gelänge, von den bis jetzt bekannten Arten künstlich in ihrer stickstoffbindenden Kraft gestärkte Rassen zu züchten, deren angezüchtete Fähigkeiten nicht nur unter bestimmten Versuchsbedingungen im Laboratorium, sondern auch im Boden konstant erhalten blieben. Diesem letzteren Problem der *Zuchtling auf Leistung*, zu dessen Verwirklichung uns zwar bis jetzt in der Boden-Bakteriologie jede Unterlage fehlt, und für das wir auf diesem Gebiet noch keine Analogien kennen, sollte man trotz alledem ernste Beachtung schenken. Durch besondere Reizmittel wie z.B. Milch- oder Flusssäure (die erstere produziert durch Milchsäurebakterien) spornt man z.B. in den Brenneibetrieben bestimmte Heferassen zu höchsten Leistungen bezüglich der Alkoholerzeugung an; ob es möglich sein wird, auch bei gewissen Azotobakterarten durch "Reizstoffe", die nur für diese Organismen spezifisch, im übrigen ziemlich indifferent wären, eine merkbare Erhöhung der Stickstoffbindung zu erreichen, darüber lässt sich heute weder im bejahenden noch verneinenden Sinne etwas aussagen. Versuche von Greaves (25) haben gezeigt, dass durch sehr geringe Zusätze von Arsen zum Boden die Stickstoffbindung sich erhöhen liess; das Arsen soll hier einerseits eine Entwicklungshemmung von dem Azotobakter schädlichen Bodenorganismen verursachen, andererseits dem Azotobakter selbst eine ökonomischere Verwertung der Kohlenstoffquellen ermöglichen. Es bleiben weitere Versuche darüber abzuwarten.

Was hinsichtlich der Leistungssteigerung über Azotobakter gesagt ist, käme auch für die sporenbildenden Stickstofffixierer in Frage. Dass mit der gesteigerten Wachstumsfreudigkeit dieser letzteren Bakteriengruppen eine erhöhte Buttersäurebildung verbunden ist, dürfte kaum für das Pflanzenwachstum von Schaden sein, denn die freie Buttersäure, die als solche zwar auf die Kulturpflanzen stark giftig wirkt (80), wird sich im Erdboden jedenfalls nicht anhäufen, sondern sofort beim Entstehen von anderen Mikroorganismen abgesättigt oder verbraucht werden.

### SCHLUSSBETRACHTUNG

Obwohl auf keinem engeren Teilgebiet der Bodenbakteriologie bis jetzt eine so grosse Zahl von Arbeiten vorliegt, wie über die stickstoffbindenden Bakterien, so wird es denn noch, wie ich durch meine vorstehenden Ausführungen gezeigt zu haben glaube, noch vieler und eingehender Studien bedürfen bis der hier behandelte Fragenkomplex restlos geklärt ist.

## ZITIERTE LITERATUR

- (1) Allen, E. R. 1920. *Ann. Missouri Bot. Gard.* 7: 75.
- (2) Ashby, S. F. 1907-8. *Jour. Agr. Sci.* 2: 35.
- (3) Barthel, Chr. 1926. *Meddel. Centralanst. Försöksv. Jordbruksomradet.* No. 308. *Bakter. avdeln* No. 43; ref. *Centbl. Bakt.* II. Abt. 69: 268.
- (4) Behrens, J. 1901. *Arb. Deut. Landw. Gesell.*, Heft 64: 108.
- (5) Beijerinck, M. W. 1891. *Verslag. Mededeel. Akad. Wetensch. Amsterdam.* Afd. Natuurk. III. 8: 460.
- (6) ———. 1918. *Proc. Sect. Scienc. Kon. Akad. Wetensch. Amsterdam.* 21: 183.
- (7) ———, und Delden, A. van. 1902. *Centbl. Bakt.* II. Abt. 9: 3.
- (8) Benecke, W., und Keutner, J. 1903. *Ber. Deut. Bot. Gesell.* 21: 333.
- (9) Bialosuknia, W., und Klott, C. 1923. *Lab. Landw. Bakt. Staatinst. Landw. Bydgoszez. Roczniki Nauk. Rolniczych.* 9: 288; ref. *Ber. Gesam. Physiol. u. Expt. Pharmacol.* 29: 141 (1925).
- (10) Bonazzi. 1915-21. *Jour. Agr. Research [U. S.]* v. 4 und 1921. *Jour. Bact.* 6: 331.
- (11) Bredemann, G. 1909. *Centbl. Bakt.* II. Abt. 22: 44.
- (12) ———. 1909. *Ibid.* 23: 385.
- (13) Brown, P. E., and Stallings, J. H. 1921. *Soil Sci.* 12: 365.
- (14) Bryan, O. C. 1922. *Ibid.* 13: 271.
- (15) Christiansen-Weniger, Fr. 1923. *Centbl. Bakt.* II. Abt. 58: 41.
- (16) Ehrenberg, P. 1925. *Ztschr. Pflanzenernähr. u. Düngung.* A5: 104.
- (17) Fischer, H. 1916. *Fühling's Landw. Ztg.* 1916: 393.
- (18) Fred, E. B. 1908. *Virginia Agr. Expt. Sta. Rept.* p. 132; ref. *Expt. Sta. Record* 21: 420 (1910).
- (19) Gainey, P. L. 1918-25. *Jour. Agr. Research* 14: 205; *Science* 48: 139; 1922, *Science* 56: 21; *Abs. Bact.* 6: 14; 1923, *Jour. Agr. Research* 24: 289 u. 907; und 1925, *Soil Sci.* 20: 73.
- (20) Gainey, P. L., und Batcheler. 1923. *Jour. Agr. Research* 24: 759.
- (21) Gautier, und Drouin. 1888-92. *Compt. Rend. Acad. Sci. [Paris]* 106: 160; 1891, *Ibid.* 113: 820; and 1892, *Bull. Soc. Chim. France* (3) 7-8: 53.
- (22) Gerlach, und Vogel. 1902-3. *Centbl. Bakt.* II. Abt. 9: 817; und 1903, *Ibid.* 10: 636.
- (23) Gillespie, L. J. 1918. *Science* 48: 393.
- (24) Golding. 1905. *Jour. Agr. Sci.* 1: 59.
- (25) Greaves, J. E. 1916. *Jour. Agr. Research [U. S.]* 6: 369.
- (26) ———. 1918. *Soil Sci.* 6: 163.
- (27) Greigh-Smith, R. 1906. *Proc. Lin. Soc. N. S. Wales* 31: 608; ref. *Expt. Sta. Record* 20: 18 (1908-9).
- (28) Haselhoff, E., und Bredemann, G. 1906. *Landw. Jahrb.* 35: 392.
- (29) Heinze, B. 1904. *Centbl. Bakt.* II. Abt. 12: 43.
- (30) ———. 1904-6. *Ibid.* 1905. *Ibid.* 14: 9; und 1906, *Landw. Jahrb.* 35: 888.
- (31) ———. 1910. *Arb. Agr.-chem. Vers. Sta. Halle der Landw. Kammer Sachsen* 3: 106.
- (32) Hills, T. L. 1918. *Jour. Agr. Research* 12: 183.
- (33) Hiltner, L. 1900. *Arb. Biol. Abt. Kais. Gesundh.-Amt.* 1: 177.
- (34) ———. 1904-6. *Lafar, Handb. Techn. Mykologie* 3: 24.
- (35) Hoffmann, C., und Hammer, B. W. 1910. *Centbl. Bakt.* II. Abt. 28: 127.
- (36) Hunter, W. O. 1923. *Jour. Agr. Research* 24: 263.
- (37) ———. 1923. *Ibid.* 23: 665.
- (38) Johnson, A. W., und Lipman, C. B. 1922. *Agr. Sci.* 4: 397.
- (39) Koch, A. 1904-6. *Lafar, Handb. Techn. Mykologie* 3: 1.
- (40) ———, und Seydel, S. 1912. *Centbl. Bakt.* II. Abt. 31: 570.

- (41) Kostytschew, S., und Ryskaltchouk, A. 1925. *Compt. Rend. Acad. Sci. [Paris]* 180: 2070.
- (42) Kostytschew, S., Ryskaltchuk, A., und Schezowa, O. 1926. *Hoppe-Seyler's Ztschr. Physiol. Chem.* 154: 1.
- (43) Leonard, L. T. 1923. *Soil Sci.* 15: 277.
- (44) Lewis, L. L., und Nicholson, J. F. 1905. *Okla. Agr. Expt. Sta. Bul.* 68.
- (45) Lipman, C. B. 1903. *New Jersey Sta. Rept.* 24: 217; 1904, *Ibid.* 25: 237.
- (46) ———, und Teakle, L. J. H. 1925. *Soil Sci.* 19: 99.
- (47) Löhnis, F. 1905. *Centbl. Bakt. II. Abt.* 14: 594.
- (48) ———, und Smith N. R. 1916. *Jour. Agr. Research* 6: 675.
- (49) Loew, O. 1890. *Ber. Deut. Chem. Gesell.* 23: 1447 u. 3018.
- (50) Makrinoff, J. A. 1924. *Soil Sci.* 17: 31.
- (51) Mazé, P. 1897. *Ann. Inst. Pasteur* 11: 44.
- (52) Mockeridge, Fl. 1924. *Biochem. Jour.* 18: 550.
- (53) Müller, A., und Stapp, C. 1925. *Arb. Biol. Reichsanst. Land u. Forstw.* 14: 455.
- (54) Niemeyer, L. 1924. *Böt. Arch.* 7: 347.
- (55) Niklas, H., Poschenrieder, H., und Hock, A. 1925. *Centbl. Bakt. II. Abt.* 66: 16.
- (56) Perkins, A. T. 1925. *Jour. Agr. Research* 30: 243.
- (57) Petersen, E. J. 1925. *Tidsskr. Planteavl.* 31: 246.
- (58) Pringsheim, H. 1911. *Biol. Zentbl.* 31: 65.
- (59) Reinke. 1903. *Ber. Deut. Bot. Gesell.* 21: 371.
- (60) Rippel, A. 1927. *Ztschr. Pflanzenernähr. Düngung u. Bodenk.* A8: 268.
- (61) Singh, Th. M. 1920. *Soil Sci.* 9: 437.
- (62) Stapp, C. 1924. *Centbl. Bakt. II. Abt.* 61: 276.
- (63) ———, und Ruschmann, G. 1924. *Arb. Biol. Reichsanst. Land u. Forstw.* 13: 305.
- (64) Stevens, J. W. 1923. *Jour. Infect. Diseases* 33: 557; ref. *Centbl. Bakt. II. Abt.* 63: 510.
- (65) Stoklasa, J., und Kricka. 1924. *Centbl. Bakt. II. Abt.* 61: 298.
- (66) ———, Ernest, Straňák und Vitek. 1906. *Ber. Deut. Bot. Gesell.* 24: 22; ref. *Centbl. Bakt. II. Abt.* 21: 484.
- (67) Strowd, W. H. 1921. *Soil Sci.* 11: 123.
- (68) Vageler, P. 1908. *Die Wissenschaft*, Heft 26.
- (69) Waksman, S. A. 1918. *Science* 48: 653.
- (70) ———, und Karunakar. 1924. *Soil Sci.* 17: 379.
- (71) Warburg, O. und Naeglelin. 1920. *Biochem. Ztschr.* 110: 66.
- (72) Whiting, A. L. und Hansen, R. 1920. *Soil Sci.* 10: 291.
- (73) ———, und Schoonover, W. R. 1920. *Ibid.* 411.
- (74) Winogradsky, S. 1893–1902. *Compt. Rend. Acad. Sci. [Paris]* 116: 1385; 1894, *Ibid.* 118: 353; 1895, *Arch. Sci. Biol. [Leningrad]* 3: No. 4; 1902, *Centbl. Bakt. II. Abt.* 9: 43.
- (75) ———. 1926. *Ann. Inst. Pasteur* 40: 455.
- (76) Wright, W. H. 1925. *Soil Sci.* 20: 95.
- (77) ———. 1925. *Ibid.* 131.
- (78) Wunschik, H. 1925. *Centbl. Bakt. II. Abt.* 64: 395.
- (79) Yamagata, U., und Itano, A. 1923. *Jour. Bact.* 8: 521.
- (80) 1923. Siehe Eddelbuttel, in Falck, *Mykologische Untersuchungen u. Berichte* 1: 256.

# THE COCCOID PHASE OF *BACILLUS AMYLOBACTER*, A. M. AND BREDEMANN

A. CUNNINGHAM AND H. JENKINS

*Edinburgh and East of Scotland College of Agriculture, Scotland*

## INTRODUCTION

The recent work of Löhnis and Smith (2, 3), Mellon (4, 5) and others on the bacteria indicates that the life-cycles of certain of these organisms may be more complex than has hitherto been supposed and that many so-called species are probably merely phases in the development of particular organisms. Life-cycle studies are therefore of fundamental importance from the point of view of systematic bacteriology and promise to simplify considerably a branch of the subject which is at present in a state of well-nigh hopeless confusion. There are indications too that work of this nature will probably be of great value in the solution of many of the problems of applied bacteriology, e.g. the bacteriology of soils. Thus Löhnis and Smith have shown that while the large-cell phase of *Azotobacter* fixes nitrogen readily most of the other phases in the life-cycle of that organism are incapable of assimilating free nitrogen. A study of the conditions which favor transformations from one phase to another might therefore be expected to yield interesting and valuable results.

With these facts in mind an investigation of the life-cycle of *B. amylobacter* was undertaken. A number of scattered observations exist in the literature with regard to the coccoid phase of this organism but Bredemann (1) is the only worker who has succeeded in obtaining it in pure culture. Bredemann's work, however, was mainly of a preliminary nature and in the first place therefore it seemed advisable to attempt to confirm or disprove his claims. An account of the investigations carried out for this purpose is given in this paper which also contains a description of the cocci obtained in the course of the work.

## EXPERIMENTAL

Five strains of *B. amylobacter* were isolated from five different soil samples by preliminary culture in dextrose solution followed by anaerobic plating on dextrose agar. About 1 g. of soil was added to each of a number of tubes of dextrose solution. The medium was then heated at 80° C. for 10 minutes, cooled and incubated anaerobically in a McIntosh and

Fildes jar at 37° C. As a rule vigorous gas formation occurred after 48 hours incubation. Tubes of melted dextrose agar were then inoculated from fermenting dextrose solutions. The medium was heated at 80° C. for 10 minutes, poured into Petri dishes and incubated anaerobically in McIntosh and Fildes jars at 37° C. After 48 hours incubation growth appeared on the plates in the form of greyish-white granular surface colonies and opaque white feathery deep colonies. The growth from selected colonies was purified by repeated plating when the cultures were transferred to milk containing calcium carbonate, heated at 80° C. for 10 minutes and kept anaerobically. They were transferred to fresh calcium carbonate-milk once a month. A sixth strain was isolated from milk.

Coccus colonies were first observed in Petri dish cultures from two strains which had already been plated four times in the course of purification. They were porcelain-white, uniform and round and were irregularly distributed in groups on the plates. When they were replated it was found that growth took place more vigorously aerobically than under anaerobic conditions. The cultures were therefore purified by repeated aerobic plating on dextrose agar. The coccus cultures are described at the end of this paper.

Many bacteriologists are of the opinion that anaerobes are exceptionally difficult to obtain in pure culture by plating methods. In fact the discrepancies in the published descriptions of so-called species of these organisms are often attributed to the prevalence of impure cultures. In the first place, therefore, it appeared to be essential to determine whether the coccus was a contaminant occurring in the cultures of the bacillus. An attempt was made to obtain single-cell cultures of the bacillus by Burri's Indian Ink method but although a considerable number of single cells was obtained in no case was it found possible to secure growth from the cells so isolated. Further plating was therefore resorted to but the coccus could still be obtained on plates from cultures which had already been plated twenty or more times.

The cultures employed in a number of the platings were submitted to a thorough microscopic examination in an attempt to discover whether the coccus was present in them. The inoculum to be used for plating was suspended in sterile water and a quantity corresponding to three times the amount used for inoculation of each plate was spread on a slide, stained with dilute fuchsin and thoroughly searched. Typical cocci were never observed in these preparations. Frequently, however, spherical or ovoid bodies attached laterally or terminally to the cells of the bacilli were seen. Similar bodies have been described by Löhnis and Smith as gonidia. When they become detached from the rods they bear a certain resemblance to cocci but as a rule they occur singly and in no case do they show the appearances which will be described later as typical of coccus cultures when first formed from the bacillus. Further, although



the coccus grows luxuriantly under aerobic conditions, such cultures when transferred to fresh media and incubated aerobically have invariably yielded no growth. It is concluded therefore that the coccus does not arise directly from these gonidia.

The plates on which cocci were first observed had been incubated in a jar in which, by accident, strictly anaerobic conditions were not secured. Cocci have never appeared on plates which have been incubated under strictly anaerobic conditions unless these have been kept aerobically for some time after removal from the McIntosh and Fildes jars. As aerobic cultures of the bacillus produced no growth it seemed probable that the coccus might be obtained when cultures were incubated under partially-aerobic conditions. This view was confirmed by means of carefully controlled experiments in which media inoculated with equal quantities of cultures of the bacillus were poured into Petri dishes and incubated at 37° C.: (1) aerobically, (2) anaerobically, (3) under partially-aerobic conditions secured by placing the cultures in a Bulloch's or McIntosh and Fildes jar and exhausting it by means of a water pump. The aerobic cultures yielded no growth, the anaerobic cultures produced the bacillus only while the plates incubated under partially-aerobic conditions frequently showed cocci.

The fact that the aerobic plates remained sterile points to the absence of the coccus as a contaminant in the bacillus cultures. Nevertheless the possibility remains that the coccus although present in the aerobic cultures was prevented from developing in some way by the presence of the bacillus. A small quantity of growth from a coccus culture was added to a culture of the bacillus and the mixed culture was plated and incubated aerobically. No evidence of inhibition of the growth of the coccus could however be obtained as numerous coccus colonies appeared on the plates even after 24 hours' incubation.

The thermal death points of the coccus and bacillus were determined and found to be 60° C. (10 minutes) and 100° C. (3 minutes) respectively. Bacillus cultures were therefore heated at 80° C. for 10 minutes, plated and incubated under partially-aerobic conditions. Cocci were obtained in a large number of these experiments while plates inoculated from the same heated cultures and incubated aerobically yielded no growth. In this connection it may be mentioned that the stock cultures of the bacillus have been transferred to fresh media twelve or more times and each time the inoculum has been heated at 80° C. Yet the cultures when placed under suitable conditions still yield cocci. It is therefore concluded that the coccus does not exist as a contaminant in the bacillus cultures.

Reference has already been made to the fact that the coccus colonies when first observed occurred in groups distributed irregularly over plates which had been inoculated with the bacillus. Similar appearances have

been frequently observed in the course of the subsequent work. Examination of the colonies reveals the fact that the majority though not all occur on the surface of the medium. It is believed that many of the surface colonies are formed as a result of small quantities of "condensation" water getting on to the surface of the medium owing to changes in pressure within the jar during incubation. This would account for the fact that a large number of the colonies are on the surface of the medium and that they are irregularly distributed on the plates. Similar appearances can be produced by the inoculation of the "condensation" water in the lid of a Petri dish with a pure culture of the coccus and incubation in a jar which has been exhausted at the pump.

Colonies of the coccus generally appear on plates which have been incubated for from two to six days at 37° C. and they are as a rule unaccompanied by colonies of the bacillus. If colonies of the bacillus are formed on a plate within the first few days of incubation cocci do not as a rule develop later. Many of the coccus colonies contain a few bacilli.

In order to control the possibility of contamination from external sources as well as to check the sterility of the media and apparatus each inoculated plate in the experiments already described was incubated along with a similar but uninoculated control plate. Cocci were obtained in a considerable number of those experiments in which the controls remained sterile. Contamination of the controls on the rare occasions on which it occurred did not generally amount to more than one or two colonies whereas from 50 to 150 or more coccus colonies have been observed on the inoculated plates.

The cocci isolated in these experiments were obtained from all six strains of the bacillus studied and were uniform in type except in the following particulars:

(1) *Gelatine liquefaction*.—The majority of the cultures fail to liquefy gelatine: a few produce a slow liquefaction. These variations are not however significant as the liquifying strains are gradually losing the ability to produce liquefaction.

(2) *Curdling of milk*.—Some strains curdle milk after several days' or weeks' incubation; others fail to curdle milk even after one month's incubation at 37° C. Again the variation is not a significant one as the strains which at first curdled milk are gradually losing this property.

(3) *Color*.—The majority of the strains examined are white. Two orange strains, culturally identical with the white forms, have been encountered. This result is in agreement with that of Löhnis and Smith who, in their studies on *Azotobacter*, obtained white and yellow cocci which were culturally identical.

The fact that the cocci obtained are all of the same type taken in conjunction with the results of the controlled experiments already described indicates that the cocci are not contaminants derived from the media or other external sources.

## DESCRIPTION OF THE CULTURES

### THE BACILLUS

The characteristics of the cultures isolated were as follows: the organism was a straight rod with rounded ends, 3–8  $\mu$  long by 1  $\mu$  broad. It was actively motile; motility could be demonstrated in a drop of liquid medium examined under a cover glass on an ordinary slide. The organism was Gram positive and produced an ovoid central or subterminal spore, usually wider than the cell. It grew well at 30 to 37° C. and slowly at 22° C. and was destroyed by boiling for 3 minutes in dextrose bouillon. It was a strict anaerobe.

On ordinary meat-extract agar minute whitish colonies were produced. Growth was more vigorous on 0.5 per cent dextrose agar and was accompanied by splitting of the medium due to gas formation. The colonies were greyish and translucent.

On ordinary meat-extract gelatine no growth was obtained, but on 0.5 per cent dextrose gelatine greyish granular colonies with hair-like outgrowths were produced. The gelatine was not liquefied.

In bouillon a very scanty growth was observed, while in 1 per cent dextrose bouillon growth was vigorous and was accompanied by gas formation.

In milk an acid frothy curd was formed; the curd contracted and expressed a considerable quantity of whitish whey.

On potato, growth was abundant and white.

All strains fermented dextrose, levulose, galactose, saccharose, maltose, lactose, xylose, dextrin, and salicin dissolved in peptone water, producing acid and gas in each case. Dulcitol and adonitol were not fermented.

### THE COCCOID PHASE

In pure culture the cocci occurred singly, in pairs, in fours, in short chains or in irregular groups. The cells occurring in pairs or fours were frequently bean-shaped. When the cocci were first formed from the bacillus they were frequently arranged in pairs or tetrads or in chains of pairs or tetrads. The chains in some cases exhibited a peculiar and characteristic branched arrangement similar to that shown by Brede-mann in the plates at the end of his paper. They generally became larger in diameter towards one end while at the opposite end one or more large undivided cocci were often found. Certain strains retained the chain arrangement persistently in pure culture. There was considerable variation in size of the cells not only amongst the different strains but also amongst the cells in any one culture. The limits of variation in diameter were 0.75  $\mu$  to 2  $\mu$ .

The organism was Gram positive, non-motile and non-sporing. It grew well at 30 to 37° C., slowly at 22° C., and not at all at 50° C., and was destroyed in dextrose bouillon at 58° to 60° C. in 10 minutes. It was a facultative anaerobe.

On ordinary agar a white growth was formed. On dextrose agar growth was abundant, opaque, porcelain-white and glistening, while the colonies were white, slightly raised and round with uniform entire margin. No gas was produced.

On ordinary gelatine a white glistening growth was formed on the surface of the medium and a greyish papillate growth in the stab. The majority of the seventeen strains examined failed to liquefy gelatine; six strains produced a very slow liquefaction.

In bouillon a dense turbidity was formed and an abundant slimy sediment accumulated at the bottom of the tube. The medium became alkaline.

Ten of the seventeen strains produced an acid-rennet curd in milk, generally after several days' incubation. A considerable quantity of a whitish whey was expressed. No gas was formed. Seven strains failed to curdle milk but rendered it slightly acid.

On potato a glistening white growth was formed and the medium was not discolored. Some strains produced a more abundant growth than others.

Acid but no gas was produced in dextrose, levulose, lactose, maltose, and saccharose dissolved in peptone water. Adonitol, inositol and inulin were not fermented even after one month's incubation.

In soil extract containing 2 per cent dextrose, 0.05 per cent dipotassium phosphate and excess of calcium carbonate no nitrogen was fixed.

#### LITERATURE CITED

- (1) Bredemann, G. 1909. Centbl. Bakt. II Abt. 23: 385.
- (2) Löhnis, F. and Smith, N. R. 1916. Jour. Agri. Research [U. S.] 6: 675.
- (3) 1923. Ibid. 23: 401.
- (4) Mellon, R. R. 1925. Jour. Bact. 10: 481, and 579.
- (5) ————. 1926. Ibid. 12: 279.

# APPLICATION OF THE SERUM-REACTION IN THE CLASSIFICATION OF AZOTOBACTER

K. ASO AND R. YOSHIDA  
*Tokyo Imperial University, Japan*

## INTRODUCTION

*Azotobacter* are generally divided into four types according to the color of pigments produced by them.

In connection with our investigation on the distribution of *Azotobacter* in the soils of Japan, we have found a special group of *Azotobacter* which produced only a light brownish color, and never got darker after a long period of culture. Consequently it seemed to us very doubtful to recognize this organism as a kind of *Azotobacter chroococcum*.

We applied the serum-reaction to distinguish various types of *Azotobacter* with success by using Wassermann's reaction (complement-fixation), and we found that the types of *Azotobacter* noted above were quite similar to *Azotobacter chroococcum*. Further we were able to distinguish clearly the other types of *Azotobacter* from each other by this serum-reaction.

## EXPERIMENT

For our experiments, we have used the following types of *Azotobacter*: *Azotobacter chroococcum* from the United States Department of Agriculture and also a strain isolated by us from the soils of Japan.

*Azotobacter beijerinckii* from the United States Department of Agriculture, and the laboratory of Prof. J. K. Wilson, Cornell University and also isolated by us from the soil of Japan.

*Azotobacter vinelandii* from the United States Department of Agriculture; and also isolated by us from the soils of Pelew and Seypan islands. The following tables show an example of the results of our experiments.

These results show that *Azotobacter chroococcum* and *Azotobacter beijerinckii* are the same kind, and it seemed to us *Azotobacter beijerinckii* is a variety of *Azotobacter chroococcum* and *Azotobacter vinelandii* is quite different from those two types.

A. Viii in the table is only a special kind of *Azotobacter* and it might be supposed that this is similar to *Azotobacter vitreum* not only from the serum-reaction but also from other facts.

TABLE 1.—Characteristics of *A. chroococcum*, *A. beijerinckii* and *A. vinelandii*Serum of *A. Ci* *Azotobacter chroococcum*

Antigen <sup>a</sup>	A. Ci <sup>o</sup>	A. Cii	A. Ciii	A. Bi	A. Bii	A. Biii	A. Vi	A. Vii	A. Viii	A. Viv
i <sup>b</sup>	++	++	++	++	++	++	++	++	++	++
ii	—	—	+	+	++	+	++	++	++	++
iii	—	—	++	++	++	++	++	++	++	++
iv	—	+	++	++	++	++	++	++	++	++
v	—	++	++	++	++	++	++	++	++	++
vi	—	++	++	++	++	++	++	++	++	++
vii	++	++	++	++	++	++	++	++	++	++
viii	++	++	++	++	++	++	++	++	++	++
ix	++	++	++	++	++	++	++	++	++	++
x	—	—	—	—	—	—	—	—	—	—

<sup>a</sup> Antigen—A. Ci, A. Cii, A. Ciii: *A. chroococcum*; A. Bi, A. Bii, A. Biii: *A. beijerinckii*; A. Vi, A. Vii, A. Viii, A. Viv: *A. vinelandii*.

<sup>b</sup> No. of test tube. i, vii, viii, ix, x all controls.

<sup>o</sup> Key: dissolved ++; slightly dissolved +; undissolved —.

TABLE 2.—Characteristics of *A. chroococcum*, *A. beijerinckii* and *A. vinelandii*Serum of *A. Vi* *Azotobacter vinelandii*

Antigen <sup>a</sup>	A. Ci <sup>o</sup>	A. Cii	A. Ciii	A. Bi	A. Bii	A. Biii	A. Vi	A. Vii	A. Viii	A. Viv
i <sup>b</sup>	++	++	++	++	++	++	++	++	++	++
ii	++	++	++	++	++	++	—	—	++	—
iii	++	++	++	++	++	++	—	—	++	+
iv	++	++	++	++	++	++	—	+	++	++
v	++	++	++	++	++	++	—	+	++	++
vi	++	++	++	++	++	++	++	++	++	++
vii	++	++	++	++	++	++	++	++	++	++
viii	++	++	++	++	++	++	++	++	++	++
ix	++	++	++	++	++	++	++	++	++	++
x	—	—	—	—	—	—	—	—	—	—

<sup>a</sup> Antigen—A. Ci, A. Cii, A. Ciii: *A. chroococcum*; A. Bi, A. Bii, A. Biii: *A. beijerinckii*; A. Vi, A. Vii, A. Viii, A. Viv: *A. vinelandii*.

<sup>b</sup> No. of test tube. i, vii, viii, ix, x all controls.

<sup>o</sup> Key: dissolved ++; slightly dissolved +; undissolved —.

## CONCLUSION

We may conclude that *Azotobacter* can be classified into three types, e.g. *Azotobacter chroococcum*, *Azotobacter vinelandii* and *Azotobacter vitreum* by the serum-reaction. Further, we shall continue this investigation especially with *Azotobacter vitreum* when we are able to obtain it from America.

# SUR LA MANIÈRE DE SE COMPORTER DE L'*AZOTOBACTER CHROOCOCCUM* DANS LE LAIT

C. GORINI

*Institut Supérieur Agronomique, Milan, Italie*

L'étude approfondie de la biologie bactérienne va démontrant de plus en plus que les exigences culturelles de certaines espèces ne présentent pas cette limitation vigoureuse qu'on leur avait attribué d'abord; certainement il y a des bactéries plus ou moins faciles à cultiver; mais les incapacités de développement dans certains milieux ou dans certaines conditions deviennent de plus en plus rares et moins absolues. On pourrait tirer plusieurs exemples chez les bactéries soidisantes aërobes obligées ou anaërobes obligées, chez les ferments spécifiques de certains sucres, etc.; mais un exemple particulièrement instructif nous est fourni par l'*Azotobacter chroococcum*.

On a soutenu pour quelque temps que les cultures pures de ce fixateur d'azote étaient entravées déjà par des quantités relativement petites de composés azotés solubles; au contraire aujourd'hui on doit reconnaître avec Beijerinck que l'adjonction d'une petite dose de nitrate potassique favorise le développement, et que, si c'est vrai que l'*Azotobacter* est capable de vivre dans des milieux tout-a-fait dépourvus d'azote, toute fois ils est aussi capable d'utiliser comme source d'azote toute une série de composés azotés soit inorganiques (nitrates et sels ammoniacaux) soit organiques (asparagine, urée, peptone, etc.), pourvu qu'ils ne soient pas dans une concentration excessive et pourvu, bien entendu, qu'il soit présent aussi une bonne source de carbone (mannite, glucose, glicérinate de chaux, etc.)

Beaucoup dépend aussi des conditions d'humidité, de réaction et d'aération des milieux.

L'*Azotobacter* a besoin d'un degré suffisant d'humidité; c'est pourquoi les milieux à l'agar au 1,5-2 % se prêtent mieux que les milieux à la gélatine à 10-12 %, ces derniers présentant une surface plus sèche.

Quant à la réaction, l'*Azotobacter* est très-sensible soit à l'acidité soit à l'alcalinité; il préfère des concentrations ionidriques autour du point neutre. C'est vraisemblablement à l'inconstance de la réaction des milieux naturels qu'on doit attribuer la discordance des auteurs sur la possibilité de cultiver l'*Azotobacter* sur les pommes de terre, les betteraves, les fèves, etc.

Quant à l'aération, en considérant le besoin prononcé d'oxygène qui a l'*Azotobacter* et son incapacité à former des pellicules à la surface des liquides, on croyait absolument nécessaire de le cultiver sur la surface des milieux solides ou tout-au-moins dans des couches très-minces de milieux

liquides, ou bien en faisant passer une courante d'air à travers les liquides. Aujourd'hui pourtant on doit reconnaître que le développement est possible dans des couches assez hautes de liquides, même sans courante d'air, pourvu qu'on ajoute aux milieux ou bien des matériaux qui servent comme soutien des bactéries (papier de filtre, sable, terre d'infusoires, etc.) ou encore des substances colloïdales (allumine, fer, acide silicique, humates) qui vraisemblablement agissent non pas autant par leur composition chimique que par leur propriétés chimicophysiques en déterminant une plus grande absorption d'oxygène.

À l'appui de ces prémisses j'ai voulu expérimenter la manière de se comporter de l'*Azotobacter* dans le lait, en me servant de trois souches qui m'ont été gracieusement envoyées par la Rothamsted Experimental Station de Harpenden (Angleterre) et de deux souches que j'ai isolé du jardin de mon Laboratoire. Voici les résultats obtenus.

L'*Azotobacter* se développe bien dans le lait à la température de l'ambiant, soit en couche mince soit en couches de 5 centimètres, même sans courante d'air, vraisemblablement grâce à l'état colloïdal de la caséine. Il faut cependant que le lait soit frais, aux une réaction amphotère ou neutre, et qu'il soit stérilisé non pas à l'autoclave mais par tyndalisation à des températures non supérieures à 100° C.

Au microscope, les cellules présentent jusqu'au deuxième mois des microformes coccoides, mobiles et fournies des typiques capsules mucilagineuses; seulement dans les cultures plus anciennes on observe des formes tératologiques, allongées et gonflées, avec une structure alvéolaire et des corpuscules métachromatiques, à côté de cellules mûres, brunâtres et à parois épaisses.

Le lait se conserve longtemps inaltéré, sans aucun signe d'acidification, ce qui vient appuyer l'opinion que l'*Azotobacter* n'attaque pas le lactose. Au contraire, certaines cultures, au bout de plusieurs semaines, montrent une tendance à l'alcalinisation avec jaunissement et clarification du lait, ce qui indique une attaque très-léger de la caséine. Pourtant elles n'arrivent jamais à une vraie solubilisation pas même après un an; au bout de ce temps les cultures présentent seulement une quantité très-petite de lait qui a acquis une consistance siroppeuse; au bout de 15-18 mois les cultures sont complètement desséchées; pourtant elles sont encore vitales; en effet, si on les ravive avec une solution nutritive fraîche (solution de Beijerinck), elles sont encore à même de fertiliser des ensemencements sur gélose dextrinée, quoique le développement soit d'abord très-lent et avec une pigmentation très-faible. Cela vient confirmer la résistance de l'*Azotobacter* à la sécheresse malgré le manque de spores et d'organes de conservation bien différenciés.

En conclusion, la culture en lait se prête assez bien et est bien commode pour la conservation prolongée de l'*Azotobacter chroococcum* dans les laboratoires.



# INFLUENCE OF THORIUM AND URANIUM SALTS ON NITROGEN-FIXATION BY AZOTOBACTER

K. HIRAI

*Kyushu Imperial University, Fukuoka, Japan*

Both thorium and uranium are radioactive elements, and some studies on the influence of uranium salts upon nitrogen-fixation by *Azotobacter* have been reported, but concerning the influence of thorium as far as we know, little work has been done. The author has carried out some experiments to ascertain the influence of thorium and compare it with uranium.

## I. INFLUENCE OF THORIUM AND URANIUM OXIDES

The usual mannite solution containing the oxide in varying quantities was inoculated with *Azotobacter*, and after the prescribed days had passed the amount of fixed-nitrogen in the solution was determined by the usual method.

To make the results clear, the author used the index number (control as 100) given in the following data.

TABLE 1.—Effect of thorium and uranium oxide upon nitrogen-fixation  
(Incubated at 28° C. for 21 days)

Oxide added per liter		Nitrogen fixed in 1 l. of solution		
		A. C. <sup>a</sup>	A. B. <sup>a</sup>	A. V. <sup>a</sup>
Control	mg.	100 <sup>b</sup>	100 <sup>o</sup>	100 <sup>d</sup>
1 Th	0	126	128	95
2 Th	30	100	123	96
3 U	60	154	143	155
4 U	30	178	168	119
Control	0	100 <sup>o</sup>	100 <sup>t</sup>	100 <sup>z</sup>
5 Th	30	95	81	90
6 Th	60	68	81	93
7 U	30	153	124	159
8 U	60	167	145	
Control	0	100 <sup>b</sup>	100 <sup>i</sup>	100 <sup>j</sup>
9 Th	30	116	129	113
10 Th	60	96	121	100
11 U	30	159	157	205
12 U	60	153	162	139

<sup>a</sup> A. C., *A. chroococcum*; A. B., *A. beijerinckii*; A. V., *A. vinelandii*.

<sup>b</sup> 82.4 mg. N

<sup>o</sup> 109.8 mg. N

<sup>h</sup> 87.4 mg. N

<sup>c</sup> 79.2 Do

<sup>t</sup> 97.6 Do

<sup>i</sup> 57.4 Do

<sup>d</sup> 114.8 Do

<sup>z</sup> 79.2 Do

<sup>j</sup> 71.0 Do

TABLE 2.—Effect of thorium and uranium oxide upon nitrogen-fixation  
(Incubated at 28° C. for 21 days)

Oxide added per liter		Nitrogen fixed in 1 l. of solution		
		A. C. <sup>a</sup>	A. B. <sup>a</sup>	A. V. <sup>a</sup>
Control	mg. 0	100 <sup>b</sup>	100 <sup>c</sup>	100 <sup>d</sup>
1 Th	20	135	101	100
2 Th	30	133	104	100
3 Th	40	116	108	103
4 Th	50	108	109	103
5 Th	60	98	107	100
6 Th	70	113	78	97
7 Th	100	162	65	97
Control	0	100 <sup>e</sup>	100 <sup>f</sup>	100 <sup>g</sup>
1 U	20	124	142	120
2 U	30	142	143	158
3 U	40	149	161	198
4 U	50	171	167	209
5 U	60	186	160	216
6 U	70	186	199	216
7 U	100	213		

<sup>a</sup> A. C., *A. chroococcum*; A. B., *A. beijerinckii*; A. V., *A. vinelandii*.

<sup>b</sup> 100.6 mg. N

<sup>c</sup> 103.2 mg. N

<sup>e</sup> 118.8 Do

<sup>f</sup> 99.2 Do

<sup>d</sup> 45.6 Do

<sup>g</sup> 76.4 Do

## II. INFLUENCE OF THORIUM AND URANIUM NITRATES

TABLE 3.—Effect of thorium and uranium nitrate upon nitrogen-fixation  
(Incubated at 28° C. for 21 days)

Nitrate added per liter (as oxide)		Nitrogen fixed in 1 l. of solution		
		A. C. <sup>a</sup>	A. B. <sup>a</sup>	A. V. <sup>a</sup>
Control	mg. 0	100 <sup>b</sup>	100 <sup>c</sup>	
1 Th	3.65	113	88	
2 Th	7.30	103	97	
3 Th	14.60	115	84	
4 Th	21.90	131	87	
5 Th	29.20	126	93	
6 Th	36.50	126	99	
7 Th	43.80	131	95	
8 Th	58.40	131	99	
9 Th	73.00	130	47	
10 Th	109.50			
Control	0	100 <sup>d</sup>	-	100 <sup>e</sup>
1 U	3.65	153		112
2 U	7.30	169		118
3 U	14.60	150		117
4 U	21.90	96		123
5 U	29.20	149		130
6 U	36.50	181		141
7 U	43.80	191		166
8 U	58.40	300		150
9 U	73.00	245		157
10 U	109.50	136		170

<sup>a</sup> A. C., *A. chroococcum*; A. B., *A. beijerinckii*; A. V., *A. vinelandii*.

<sup>b</sup> 66.4 mg. N

<sup>d</sup> 23.2 mg. N

<sup>c</sup> 76.9 Do

<sup>e</sup> 18.1 Do

## III. INFLUENCE OF THORIUM SULFATE

TABLE 4.—Effect of thorium sulfate upon nitrogen-fixation

(Incubated at 28° C. for 21 days)

Sulfate added per liter (as oxide)		Nitrogen fixed in 1 l. of solution	
		A. C. <sup>a</sup>	A. V. <sup>a</sup>
Control	mg. 0	100 <sup>b</sup>	100 <sup>c</sup>
1	3.65	100	100
2	7.30	116	109
3	14.60	103	106
4	21.90	132	109
5	29.20	113	110
6	36.50	138	106
7	43.80	122	103
8	58.40	110	120
9	73.00	129	106

<sup>a</sup> A. C., *A. chroococcum*; A. V., *A. vinerandii*.<sup>b</sup> 43.8 mg. N<sup>c</sup> 47.0 Do

## IV. INFLUENCE OF URANIUM ACETATE

TABLE 5.—Effect of uranium acetate upon nitrogen-fixation

(Incubated at room temp. viz. 27–33° C. for 40 days)

Acetate added per liter (as oxide)		Nitrogen fixed in 1 l. of solution		
		A. C. <sup>a</sup>	A. B. <sup>a</sup>	A. V. <sup>a</sup>
Control	mg. 0	lost <sup>b</sup>	100 <sup>c</sup>	100 <sup>d</sup>
1	3.65	100	156	113
2	5.11	103	100	115
3	7.30	108	125	115
4	14.60		118	100
5	21.90		137	100
6	29.20	146	125	145
7	36.50	106	106	115
8	43.80	103	112	111
9	58.40	120	112	113
10	73.00	113		111

<sup>a</sup> A. C., *A. chroococcum*; A. B., *A. beijerinckii*; A. V., *A. vinerandii*.<sup>b</sup> 95.6 mg. N<sup>c</sup> 43.8 Do<sup>d</sup> 64.0 Do

## V. INFLUENCE OF URANIUM CHLORIDE

TABLE 6.—*Effect of uranium chloride upon nitrogen-fixation*

(Incubated at 28° C. for 21 days)

Chloride added per liter (as oxide)		Nitrogen fixed in 1 l. of solution		
		A. C. <sup>a</sup>	A. B. <sup>a</sup>	A. V. <sup>a</sup>
Control	mg. 0	100 <sup>b</sup>	100 <sup>c</sup>	100 <sup>d</sup>
1	3.65	132	106	112
2	7.30	211	121	109
3	14.60	195	94	100
4	21.90	217	106	147
5	29.20	222	115	173
6	36.50	238	147	153
7	43.80	222	138	182
8	58.40	275	129	146
9	73.00	204	118	179
10	109.50		136	191

<sup>a</sup> A. C., *A. chroococcum*; A. B., *A. beijerinckii*; A. V., *A. vinelandii*.<sup>b</sup> 25.4 mg. N<sup>c</sup> 45.6 Do<sup>d</sup> 45.6 Do

## VI. CHANGES IN THE NUMBER OF ORGANISMS IN THE SOLUTION

The author has also studied the changes in the number of organisms in the solution by Breed's method. The results obtained in one experiment (Table 1, Expts. 9, 10, 11, 12) are as follows:

TABLE 7.—*Changes in number of organisms as effected by uranium and thorium nitrate*

Oxide added per liter		Number of organisms in 1 c.c. solution					
		A. C.		A. B.		A. V.	
		At start	At end	At start	At end	At start	At end
Control	mg. 0	543,108.5	53,896,650.6	6,345,020.0	59,458,300.0	1,222,782.0	133,728,650.0
T	30	Do	89,017,850.0	Do	94,439,950.0	Do	259,053,300.0
T	60	Do	84,670,300.0	Do	93,494,500.0	Do	322,503,500.0
U	30	Do	106,100,500.0	Do	114,714,600.0	Do	319,352,000.0
U	60	Do	121,858,000.0	Do	212,201,000.0	Do	464,846,250.0

## SUMMARY

Uranium salt increased the nitrogen-fixation by *Azotobacter* in all cases.

The increase in fixation varied with the added quantity of uranium salt and the different organisms. For the same quantity, thorium salt had not such a good effect as uranium as far as the author's experiments are concerned. Sometimes, it not only had no influence but rather inhibited the activity of *Azotobacter*. The number of organisms also increased by the addition of these salts.

# INFLUENCE OF SOIL PROTOZOA ON NITROGEN-FIXATION BY AZOTOBACTER

K. HIRAI AND I. HINO

*Kyushu Imperial University, Fukuoka, Japan*

Free nitrogen-fixation in soils is an important process and there are many studies on the organisms causing this process. At the same time, we have little knowledge of the relation between the soil protozoa and the above organisms.

However, Nasir (Annals. App. Biol. Vol. 10, 122, 1923) has studied the influence of protozoa on Azotobacter, and from his results we can understand that Azotobacter is capable of fixing more atmospheric nitrogen in the presence of protozoa than in their absence. Recently, the authors also have carried out some experiments on this same problem and the results obtained are as follows.

## EXPERIMENTAL

In our experiments, a solution of the following composition was used as the culture solution:

Mannite 10 g., magnesium sulfate 0.2 g., sodium chloride 0.2 g., dipotassium phosphate 0.2 g., calcium sulfate 0.1 g., water (Distilled) 1000 cc.

pH of this solution was changed in various ways as indicated.

Fifty cubic centimeters of this solution was placed in a 500 cc.-Erlenmyer flask and after sterilization allowed to cool, the desired quantity of Azotobacter suspension and Ciliates culture then being added. In this case, only the cystic form of Ciliates was used in order to make the number of Ciliates as uniform as possible.

## EXPERIMENT 1. ARTIFICIAL CULTURE SOLUTION

TABLE 1.—Nitrogen fixed in artificial culture solution

(Incubated at 20–25° C. 14 days)

Organisms <sup>a</sup> present	Nitrogen fixed per gram of mannite	Gain or loss		pH of culture solution	
		Actual	Per cent	At start	At end
A	mg. 12.00			7.0	6.2
A+B	12.58				
	12.56				
	12.00	−0.10	− 0.78	7.0	6.4
	12.00				
A+C	14.24				
	14.24	+2.13	+17.33	7.0	6.8
	14.76				
C				7.0	7.2
None				7.0	7.0

<sup>a</sup> A, Means *Azotobacter vinelandii*; C, Ciliates (*Colpoda Saprophylla* Stokes 12–37 $\mu$ ); B, Bacteria isolated from Ciliate culture.

TABLE 2.—Change in the number of organisms in 1 cc. of artificial culture solution

Organisms <sup>a</sup> present	No. of protozoa		No. of bacteria	
	At start	At end	At start	At end
A			2	379
A+B			13	26,550
A+C	21	8790(1110) <sup>b</sup>	42	29,510
C	21	660(30)	40	5050

<sup>a</sup> Same as Table 1.

<sup>b</sup> The figures in brackets give the active form of Ciliates, others the total number of Ciliates.



## EXPERIMENT 2. ARTIFICIAL CULTURE SOLUTION

TABLE 3.—Nitrogen fixed in artificial culture solution

(Incubated at 20–25° C. 14 days)

Organisms <sup>a</sup> present	Nitrogen fixed per gram of mannite	Gain or loss		pH of culture solution	
		Actual	Per cent	At start	At end
A	mg. 7.51			6.6	6.0
	7.51				
	7.70			6.0	5.8
A+B	7.42				
	7.42				
A+C	8.19				
	8.88	+1.03	+13.71	6.6	6.3
C				6.6	6.8
B				6.6	6.6
None				6.6	6.6

<sup>a</sup> Same as Table 1.

## EXPERIMENT 3. ARTIFICIAL CULTURE SOLUTION

TABLE 4.—Nitrogen fixed in artificial culture solution

(Incubated at 20–25° C. 14 days)

Organisms <sup>a</sup> present	Nitrogen fixed per gram of mannite	Gain or loss		pH of culture solution	
		Actual	Per cent	At start	At end
A	mg. 5.47				
	5.94			6.2	6.0
	5.00				
A+B	5.47				
	5.00			6.2	5.8
	5.94				
A+C	5.94				
	5.94	+0.61	+11.15	6.2	6.2
	6.37				
C				6.2	6.4
B				6.2	6.2
None				6.2	6.2

<sup>a</sup> Same as Table 1

## EXPERIMENT 4. ARTIFICIAL CULTURE SOLUTION AND CALCIUM CARBONATE

TABLE 5.—Nitrogen fixed in artificial culture solution and 5 g. of calcium carbonate  
(Incubated at 20–25° C. 14 days)

Organisms <sup>a</sup> present	Nitrogen fixed per gram of mannite	Loss or gain		pH of culture solution	
		Actual	Per cent	At start	At end
A	mg. 6.48 7.10 7.10			7.0	7.0
A+B	7.10 6.28 7.10	−0.07	−1.02	7.0	7.0
A+C	8.74 8.74 9.38 8.19	+1.87	+28.59	7.0	7.6
A+C+B f	8.18 8.18	+1.29	+18.12	7.0	7.4
A+P	7.64 8.18 8.18	+1.11	+16.11	7.0	7.4
C				7.0	8.0
None				7.0	7.0

<sup>a</sup> Same as Table 1; with the addition of P indicating all protozoa isolated from the soil, and B. f. indicating pure culture of *B. fluorescens*.

TABLE 6.—Change in the number of organisms in 1 cc. of artificial culture solution and calcium carbonate

Organisms <sup>a</sup> present	No. of protozoa		No. of bacteria	
	At start	At end (thousands)	At start (thousands)	At end (millions)
A			0,073	37,510
A+B			312,818	189,365
A+C	8	43.2	671,662	98,800
A+C+B f	8	16.4	775,563	186,945
A+P	4	42.2	644,191	185,130
C				
None				

<sup>a</sup> Same as Table 5.

## EXPERIMENT 5. SAND CULTURES

In these sand cultures, 100 g. of well cleaned sand was placed in a 500 cc. covered glass tumbler and its water holding capacity was made up by adding the culture solution used for the above experiments. After two or three days of *Azotobacter* inoculation, *Ciliates* culture was added to obtain a vigorous growth, Microscopic examination was carried out at the intervals of the seventh and fourteenth days to ascertain whether the protozoa were growing or not, and the protozoa were seen growing in an active state. In these cultures, part of the sand surface was browned and some was not, but in regard to quantity of fixed-nitrogen we could see no difference between them.

TABLE 7.—*Nitrogen fixed in pure sand cultures*  
(Incubated at 20–25° C. 21 days)

Nitrogen fixed in 100 g. sand *		Gain or loss	
Azotobacter	Azotobacter and Ciliates	Actual	Per cent
mg.	mg.	mg.	mg.
17.78	20.79	+4.58	+23.25
14.76	19.45	+3.24	+19.32
16.10	21.46	+5.25	+32.38
	22.13	+5.02	+37.07
	19.45	+3.24	+19.32
	21.46	+5.25	+32.38
	18.76	+2.57	+15.85

\* Sand saturated with 80 per cent of its water holding capacity.

## EXPERIMENT 6. SAND CULTURES

TABLE 8.—*Nitrogen fixed in pure sand cultures*  
(Incubated at 20–20° C. 21 days)

Nitrogen fixed in 100 g. sand *		Gain or loss	
Azotobacter	Azotobacter and Ciliates	Actual	Per cent
mg.	mg.	mg.	mg.
18.11	19.45	+1.34	+7.40
18.78	18.78	+0.67	+3.70
17.43	21.46	+3.35	+8.50
	17.43	−0.68	−3.75
	16.77	−1.34	−7.40
	18.11	±0.00	+0.00
	18.11	±0.00	+0.00
	18.78	±0.67	+3.70

\* Same as Table 7.

## SUMMARY

Nitrogen-fixation by *Azotobacter* is generally stimulated and not inhibited in the presence of soil protozoa.

In the presence of soil protozoa, the highest fixation of nitrogen recorded in these experiments is 37.07 per cent over the control plot in a sand culture. Out of 15 experiments 11 showed a decided gain in nitrogen-fixation over the control and 2 showed no gain, while 2 gave negative results, and this only in cases where saturated sandy soil is used.

The pH value of the nutrient media always changes in the course of experiments; when *Azotobacter* alone exists it slightly acidifies, while with soil protozoa alone it alkalifies in all cases.

It seems to the authors that the soil protozoa and *Azotobacter* live in a state of disjunctive symbiosis or of metabiosis in the strict sense; in other words, the presence of soil protozoa decreases the acidity of the nutrient media, resulting in vigor of growth and increased fixation of *Azotobacter*, consequently having a favorable effect on the protozoa themselves increasing their vigor and prolificity, thereby preserving the active state of the protozoa for a longer period.

# STUDIES ON NITROGEN-FIXATION BY INOCULATED SOYBEANS

L. W. ERDMAN AND J. M. FIFE  
*Iowa State College, U. S. A.*

## INTRODUCTION

It is commonly accepted that inoculated soybeans when grown under favorable soil and climatic conditions can utilize the free nitrogen of the atmosphere. There is some data showing a definite fixation of nitrogen from the air due to the inoculation of soybeans. The amount of fixation is usually calculated by determining the difference between the amount of nitrogen present in inoculated and uninoculated plants. In practically all cases where reports have been made on nitrogen-fixation by soybeans, the data presented show only the amount fixed by *inoculated* soybeans. No mention is made of differences in nitrogen-fixation in the case of soybeans showing varying degrees of inoculation. Analyses for nitrogen have been made only on inoculated and uninoculated plants, no attempt being made to separate the inoculated plants into different groups on the basis of the number and size of the nodules present on the roots.

In the soybean inoculation experiments which have been carried out at the Iowa Agricultural Experiment Station, it has been repeatedly observed, year after year, that soybean plants, inoculated by different methods, using both soil and cultures of soybean bacteria, have consistently shown varying degrees or intensities of nodulation. These observations have raised the interesting question, "What degree of inoculation on soybeans must be secured before any appreciable quantities of nitrogen are fixed from the atmosphere?" The purpose of this paper is to present some experimental data that throw some light on this question.

## MATERIALS AND METHODS

For the investigation samples of Manchu soybean plants were taken from 3 plots in the 1925 soybean inoculation field experiments. These plots were treated as follows:

Plot No.	Inoculation and Soil Treatment
1	Soybean seed inoculated with soil.
2	Soybean seed inoculated with soil, 500 lb. hydrated lime per acre.
3	Soybean seed inoculated with soil, 500 lb. hydrated lime per acre, 200 lb. acid phosphate per acre.

When the soybeans had reached the hay stage, 100 representative plants were carefully dug from each plot. The roots and nodules were thoroughly washed free of soil, and the plants from each plot were separated into 5 classes depending upon the number and size of the nodules as follows:

Class 0—No nodules present on the roots.

Class 1—Plants with one or two small or medium sized nodules.

Class 2—Plants with one or two large nodules, or in addition one or two small or medium sized nodules, or three or four small or medium sized nodules.

Class 3—Plants with three or four large nodules, or five to nine small or medium sized nodules.

Class 4—Plants with three or more large nodules, and in addition three or more small or medium nodules, or ten or more nodules of any size.

These samples were allowed to become thoroughly air-dry, after which a record was made of the total number and weight of the plants in each class. Later the plants were divided into tops and roots and the weights of these portions were recorded. The tops and roots from 10 representative plants in each class were ground up very fine in a plant grinding machine, and these samples were used for the nitrogen determinations which were made by the modified Gunning method.

In calculating the amount of nitrogen fixed per acre by the plants in each class, it was first necessary to determine the yield per acre per class. This was found by multiplying the total yield of each plot per acre by the per cent of the total yield for each class. Then the yield per acre for each class was multiplied by the per cent of nitrogen found in the uninoculated plants. By subtracting this result from the total amount of nitrogen found in the plants from each class, the amount of nitrogen fixed per acre per class was finally obtained.

## EXPERIMENTAL DATA

The results of this study showing the correlation between the degree of nodulation and the amount of nitrogen fixed from the air by Manchu soybeans are presented in Table 1.

The per cent of total nitrogen in the tops of Manchu soybeans inoculated with soil gradually increased with increasing intensity of inoculation until Class 4 was reached where the per cent of nitrogen was practically the same as that noted for Class 3. With the roots of these plants the difference in the per cent of nitrogen between the plants showing different degrees of nodulation was much greater than in the case of the tops. This was to be expected, however, since the nodules on soybeans are relatively high in nitrogen, often containing from 6.0 to 8.0 per cent nitrogen.

Great variation was obtained in the yield per acre of the soybean tops and roots in the different classes. The majority of the plants on Plot 1 were placed in Classes 2 and 3. This fact also resulted in a variation

TABLE 1.—Showing the correlation between the degree of nodulation and the amount of nitrogen fixed from the air by inoculated Manchu soybeans

Class No.	Tops			Roots			Tops and roots			
	Yield per acre per class	Total nitrogen per cent	Total nitrogen lb. per A.	Nitrogen fixed per A. lb.	Yield per acre per class	Total nitrogen per cent	Total nitrogen lb. per A.	Nitrogen fixed per A. lb.	Yield per acre per class	Total nitrogen lb. per A.
Plot 1—Manchu soybeans inoculated with soil										
0	665	1.41	9.38		107	0.82	0.66		772	1.30
1	408	1.45	5.95	0.19	57	0.80	0.45	0.10	465	1.37
2	1148	1.65	19.00	2.80	212	1.28	2.71	1.39	1360	1.59
3	1685	1.79	32.10	8.30	293	1.31	3.82	1.99	1978	1.72
4	430	1.78	7.65	1.58	71	1.64	1.17	.73	501	1.76
Total	4336		74.08	12.87	740		8.81	4.21	5076	82.89
Plot 2—Manchu soybeans inoculated with soil plus lime										
0	308	1.67	5.15		34	0.69	0.23		342	1.57
1	559	1.77	10.45	1.13	65	0.93	0.60	0.13	625	1.67
2	900	2.00	18.00	3.05	126	1.42	1.77	0.90	1026	1.92
3	2562	1.99	51.30	8.60	432	1.71	7.40	4.53	2994	1.95
4	856	2.19	18.80	4.41	130	1.91	2.48	1.59	986	2.15
Total	5185		103.70	17.19'	787		12.48	7.15	5973	116.18
Plot 3—Manchu soybeans inoculated with soil plus lime plus acid phosphate										
0*	438	1.71	7.52	0.20	68	0.78	0.53	0.06	506	1.60
1	890	1.95	17.35	2.48	150	1.29	1.93	0.90	1040	1.91
2	1848	2.00	36.96	6.06	357	1.52	5.43	2.88	2205	1.94
4	2068	2.11	43.70	9.10	464	1.73	8.02	4.82	2532	2.05
Total	5244		105.53	17.84	1039		15.91	8.66	6283	122.62
										26.50

\* Increases due to fixation have been calculated using the analyses for the uninoculated plants receiving the lime treatment.

in the total amount of nitrogen present in the soybeans from the different classes, as well as the amount of nitrogen fixed per acre per class. The greatest fixation of nitrogen from the atmosphere took place in the plants of Class 3. With the tops this amounted to 8.3 lb. per acre, while with the roots approximately 2 lb. of nitrogen were taken from the air.

The relative amount of nitrogen in the tops and roots which was fixed from the air was governed very largely by the degree of nodulation on the plants. When the figures for Plot 1 showing the total amount of nitrogen fixed by all classes are considered, 75.3 per cent of the nitrogen fixed was found in the tops and 24.7 per cent was found in the roots. But when individual classes are considered, the per cent of nitrogen which was fixed in the tops and roots was changed considerably as shown by the following data:

- Class 1—65.5 per cent of nitrogen fixed in the tops, and  
34.5 per cent in the roots.
- Class 2—66.8 per cent of nitrogen fixed in the tops, and  
33.2 per cent in the roots.
- Class 3—80.6 per cent of nitrogen fixed in the tops, and  
19.4 per cent in the roots.
- Class 4—68.4 per cent of nitrogen fixed in the tops, and  
31.6 per cent in the roots.

Lime increased the degree of inoculation very much as shown by the greater number and yield of plants in Classes 3 and 4. Lime also increased the per cent of nitrogen in the tops of soybean plants very materially over the unlimed plants, and again the percentage of nitrogen increased with increasing intensity of inoculation. The difference in favor of the slightly inoculated plants represented by Class 1, amounted to only 0.1 per cent, but in the case of the best inoculated plants, represented by Class 4, the difference amounted to 0.52 per cent. The soybean plant tops from Class 3 contained practically the same per cent of nitrogen as those in Class 2.

Both lime and inoculation increased the per cent of nitrogen in the roots of soybeans, but lime without inoculation had but little effect on the nitrogen content of soybean plant roots. The greatest increase in per cent of nitrogen in the roots of the limed plants was found in the best inoculated plants, the difference in favor of inoculation over the uninoculated plant roots being 1.22 per cent.

Lime also seemed to increase the amount of nitrogen which was fixed in the roots of soybeans but decreased the amount which was fixed in the tops when the data are considered for all of the classes. The amount of nitrogen fixed from the air and found in the tops was 70.6 per cent, while 29.4 per cent was found in the roots. The relative amount of nitrogen fixed in the tops and roots of the plants in all classes from the limed plot is shown by the following figures:



Class 1—89.7 per cent of nitrogen fixed in tops, and  
10.3 per cent in the roots.

Class 2—77.2 per cent of nitrogen fixed in tops, and  
22.8 per cent in the roots.

Class 3—65.5 per cent of nitrogen fixed in tops, and  
34.5 per cent in the roots.

Class 4—73.5 per cent of nitrogen fixed in tops, and  
26.5 per cent in the roots.

It is interesting to note that these figures do not agree at all with those obtained from the plot receiving no lime. The plants from Classes 1, 2 and 4 of the unlimed plot had a much lower per cent of nitrogen in the tops than those from the same classes of the limed plot. The plants from Class 3, however, from the unlimed plot had 80.6 per cent of the nitrogen fixed from the air present in the tops whereas the plant tops from the same class from the limed plot had 65.5 per cent of the nitrogen fixed from the air.

When compared with the unlimed soybeans, the lime increased the amount of nitrogen fixed from the air by 7.26 lb. per acre, and of this amount, 4.32 lb. was found in the tops and 2.94 lb. was found in the roots.

The use of lime and acid phosphate increased the degree of inoculation still more than when lime was used alone. In fact all of the plants on this plot were found to be inoculated, and the great majority of the plants showed very excellent nodulation. Acid phosphate, however, did not exert any influence whatever on the percentage of nitrogen in the tops of Manchu soybeans, but seemed to decrease the percentage of nitrogen found in the roots. The analyses of the plants, both tops and roots, from Plot 3 again show that the per cent of nitrogen increased with increasing degree of inoculation. The differences due to different intensities of nodulation were not as great for the plant roots receiving lime and acid phosphate as in the case of the plant roots receiving lime alone.

As a result of the increased yield due to the lime and acid phosphate, and especially the large yield noted for the plants in Class 4, a somewhat greater amount of nitrogen, 2.16 lb., was fixed per acre by this treatment than where lime alone was used.

When the data showing the total fixation of nitrogen per acre by the plants in all classes from Plot 3 are considered, it may be noted that 67.3 per cent of the nitrogen fixed was in the tops, and 32.7 per cent of the nitrogen fixed was found in the roots. The data showing the relative amount of nitrogen fixation by the soybean plant tops and roots which were treated with lime and acid phosphate are as follows:

Class 1—76.9 per cent of nitrogen fixed in tops, and  
23.1 per cent in the roots.

Class 2—73.3 per cent of nitrogen fixed in tops, and  
26.7 per cent in the roots.

Class 3—67.8 per cent of nitrogen fixed in tops, and  
32.2 per cent in the roots.

Class 4—65.4 per cent of nitrogen fixed in tops, and  
34.6 per cent in the roots.

These data show that the per cent of nitrogen fixed decreases in the soybean plant tops and increases in the roots as the degree of nodulation on the roots is increased. The results show that soybeans offer very little promise as a soil-building crop unless they are exceptionally well inoculated, and that the degree or intensity of the inoculation on soybean roots determines to a large extent the amount of nitrogen fixed from the atmosphere. Where the plants are only slightly inoculated the amount of nitrogen fixed by soybeans is practically negligible, but when they are very well inoculated very appreciable amounts of nitrogen may be expected to be taken from the atmosphere.

### CONCLUSIONS

The following conclusions may be drawn from this study:

The per cent of nitrogen in inoculated soybean plant tops and roots increases with increasing intensity of nodulation.

The amount of nitrogen fixed from the air by inoculated soybeans is proportional to the degree of nodulation on the roots.

The per cent of nitrogen fixed by inoculated soybeans decreases in the soybean plant tops and increases in the roots as the degree of nodulation on the roots is increased.

Lime alone, and in combination with acid phosphate, increased the amount of nitrogen fixed from the atmosphere by inoculated soybeans.

# INFLUENCE OF NITRATES AND SULFATES ON THE NODULE BACTERIA AND NODULE FORMATION OF GENGE, LUPIN AND SERRADELLA

S. OHKAWARA

*Tokyo Imperial University, Japan*

Wilson found that the formation of nodules was prevented by the presence of a certain amount of nitrates and sulfates, although the bacteria retained their vitality under such condition. But it is not yet clearly understood as to the cause of this phenomenon, and I was induced to investigate this subject. I have been experimenting since last year, and this paper deals with a part of the results obtained thus far and a further report in detail will follow.

## EXPERIMENT 1.—RESISTANCE OF NODULE BACTERIA TO NITRATES AND SULFATES

(a)  $KNO_3$ ,  $NaNO_3$ ,  $Ca(NO_3)_2$  and  $(NH_4)_2SO_4$ . Bacteria used for this purpose:

Genge bacteria: Isolated from Genge nodules with soil-extract-mannit agar on June 18, 1926.

Lupin and Serradella bacteria: Isolated from Lupin and Serradella nodules with same medium as mentioned above on July 10, 1925. These three bacteria were tested for their vitality previous to this experiment.

(b) Method.

The determination of nitrogen in these salts potassium nitrate, sodium nitrate, calcium nitrate, and ammonium sulfate were made and 0.1, 0.2, 0.5, 1.0, 2.0 per cent solutions of their salts were prepared and 5 cc. of these solutions were placed in each test tube, respectively, and sterilized in the Koch's steam sterilizer for 15 minutes on 3 successive days. Then 0.1 cc. of bacterial emulsion were added to each test tube and carefully shaken.

After 14, 18, 21, 24, 27, 30, 35, and 40 days, the quantity of one platinum loopful (3mm.) was transferred to other cultural solution, and incubated for 5 days at 30° C. The results observed are as follows (Table 1).

TABLE 1.—Resistance of nodule bacteria to nitrates.

		14 days	18 days	21 days	24 days	27 days	30 days	35 days	40 days
$KNO_3$	Control	++	++	++	++	++	++	++	++
	0.1%	++	++	++	++	++	++	++	++
	0.2%	++	++	++	++	++	++	++	--
	0.5%	++	++	++	++	++	++	--	--
	1.0%	++	++	++	++	--	--	--	--
	2.0%	++	++	--	--	--	--	--	--
$NaNO_3$	0.1%	++	++	++	++	++	++	++	++
	0.2%	++	++	++	++	++	++	--	--
	0.5%	++	++	++	++	++	--	--	--
	1.0%	++	++	+	--	--	--	--	--
	2.0%	++	++	--	--	--	--	--	--
$Ca(NO_3)_2$	0.1%	++	++	++	++	++	++	++	++
	0.2%	++	++	++	++	++	++	+	--
	0.5%	++	++	++	++	++	++	--	--
	1.0%	++	++	+	+	--	--	--	--
	2.0%	++	++	+	--	--	--	--	--
$(NH_4)_2SO_4$	0.1%	++	++	++	++	++	++	--	--
	0.2%	++	++	--	+	--	--	--	--
	0.5%	++	++	+	--	--	--	--	--
	1.0%	--	--	--	--	--	--	--	--
	2.0%	--	--	--	--	--	--	--	--

From the results noted above it may be concluded that the nodule bacteria retained their vitality in the 0.1 per cent nitrates for about 35 to 40 days, and even in 1 per cent solutions about 3 weeks, but ammonium sulfate exerted rather retarding action at these concentrations.

## EXPERIMENT 2.—INFLUENCE OF NITRATES AND SULFATES ON NODULE FORMATION

(a)  $KNO_3$ ,  $NaNO_3$ ,  $Ca(NO_3)_2$  and  $(NH_4)_2SO_4$ . Bacteria used in this experiment:

Genge, Lupin and Serradella nodule bacteria of the same kind as mentioned above, were used.

(b) Preparation and treatment.

(I) Percentage of the salt solutions used, were as follows, 0.005, 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1.0. These solutions were sterilized in the Koch's steam sterilizer as before.

(II) Quartz sand was soaked in concentrated hydrochloric acid for 5 to 6 days, and was well washed with distilled water and dried in the hot-air sterilizer at 150° C.

(III) Brown colored small glass pots, 7 cm. wide and 8 cm. deep, were washed clean and dried in a hot-air sterilizer.

(IV) Seeds were steeped in mercuric chloride solution of (1:1000) for 2 to 3 minutes, and then they were washed thoroughly with sterile distilled water.

## (c) Method.

Four hundred g. of quartz sand were placed in each pot, covered with Petri dishes and sterilized for 1 hour at 180° C. in the hot-air sterilizer. Then we introduced 80 cc. of the various salt solutions, per pot, with a sterilized pipette.

The planting was carefully made in the sterilized glass box with sterilized pincette and seed inoculation was used in this case. The seeds of Lupin and Serradella were mixed and sown in each pot, because these two are the host for the same bacteria. Then all the pots were transferred into the greenhouse, covered with pouches made of parafin paper and we supplied distilled water on every third day to balance the water lost by evaporation. After 70 to 80 days, the number of root-tubercles and length of plants were determined and the results obtained were as given in Table 2.

From the results indicated we may conclude that the nodule formation of Lupin and Serradella was prevented by the presence of 0.2 per cent nitrates, but it was rather stimulated by the dilute concentration of 0.02 and 0.05 per cent. In the case of sulfate (ammonium sulfate), it was prevented by the presence of 0.1 per cent and the best concentration for the nodule formation was 0.01 and 0.02 per cent.

Lastly, I express my hearty thanks to Prof. K. Asō for his kind suggestions during this investigation.

TABLE 2.—*Influence of potassium nitrate upon plants inoculated with Lupin bacteria*

Lupin							Serratella					
Concentration	No. of seedlings	Length in average	Average length of root	No. of nodules of 1 plant	Total No. of nodules	Remarks	No. of seedlings	Length in average	Average length of root	No. of nodules of 1 plant	Total No. of nodules	Remarks
Control	2	cm. 4.5	cm. 5.0	3.5	8	Good	5	cm. 2.0	cm. 3.0	3, 2, 2, 5, 3	15	Good
	2	4.0	4.0	2, 3	5	Do	3	1.5	2.5	4, 3, 1	8	Do
0.005%	1	5.0	6.5	5	5	Best	6	3.5	4.5	4, 5, 2, 8, 6, 3	28	Best
0.01%	2	4.5	5.0	1, 3	4	Very good	6	2.5	3.0	1, 3, 2, 5, 3, 3	17	Very good
	2	4.5	5.0	3, 2	5	Do	5	2.5	3.0	4, 3, 2, 2, 3	14	Do
0.02%	2	5.0	5.5	4, 5	9	Best	5	2.5	3.5	1, 3, 3, 2, 3	12	Do
	2	6.0	6.5	6, 5	11	Do	5	3.0	5.5	3, 3, 6, 6, 5	23	Best
0.05%	2	5.3	5.0	4, 3	7	Do	4	2.5	3.5	3, 3, 2, 4	12	Very good
	2	5.0	6.0	4, 4	8	Do	4	3.5	4.0	2, 7, 6, 6	21	Best
0.1%	2	5.0	5.0	3, 5	8	Do	5	3.0	3.5	2, 2, 5, 3, 1	13	Do
	2	5.0	4.5	1, 2	3	Very good	5	3.0	3.0	4, 4, 5, 2, 1	12	Very good
0.2%	2	5.0	5.0	3, 3	6	Do	4	3.5	3.5	3, 2, 2, 1	8	Do
	2	5.0	3.5	1?	1?	Good	5	2.5	3.0	2	2	Good
0.5%	1	5.5	4.0			Bad	6	3.0	3.0			Do
	2	3.0	2.5			Do	5	2.5	2.5			Do
1.0%	1	4.0	3.0			Do	4	2.0	2.0			Bad
	1	2.0	1.5			Very bad	1	1.5	1.0			Do
	1	2.0	2.5			Do	3	1.0	1.0			Do

TABLE 3.—*Influence of potassium nitrate upon plants inoculated with Serratella bacteria*

Lupin							Serratella					
Concentration	No. of seedlings	Length in average	Average length of root	No. of nodules of 1 plant	Total No. of nodules	Remarks	No. of seedlings	Length in average	Average length of root	No. of nodules of 1 plant	Total No. of nodules	Remarks
Control	2	cm. 4.0	cm. 3.5	2, 1	3	Good	3	cm. 1.8	cm. 2.0	6, 1, 3	10	Good
	2	4.5	4.0	2, 3	5	Very good	3	2.5	3.0	5, 5, 7	17	Do
	2	5.0	5.0	4, 3	7	Do	5	3.0	4.5	4, 14, 2, 3, 3	26	Best
0.005%	1	4.5	4.0	2	2	Do	4	2.0	3.0	2, 8, 3	13	Very good
	2	5.0	6.0	5, 7	12	Best	5	3.0	4.5	5, 4, 2, 6, 3	20	Best
	2	4.0	4.5	3, 4	7	Very good	4	2.5	2.5	4, 2, 5, 1, 2	14	Do
0.02%	1	5.5	6.0	7	7	Best	3	3.0	4.5	2, 2, 4, 5, 3	16	Very good
	2	5.0	5.5	2, 2	4	Do	5	3.0	3.5	4, 7, 7, 5, 8	31	Best
	2	5.0	5.0	3, 7	10	Do	5	2.5	3.0	6, 2, 5, 4, 2	19	Do
0.05%	2	5.2	5.0	1, 5	6	Very good	5	3.0	4.0	4, 3, 2, 2, 3	14	Very good
	2	5.0	6.0	7, 4	11	Do	5	2.5	3.5	3, 1, 1, 1, 2	8	Best
	2	5.0	5.0	4, 2	6	Good	5	2.5	3.0	2, 1	3	Very good
0.1%	2	5.0	4.0	1	1	Do	5	3.0	2.5			Do
	2	4.0	3.0	1, 3	4	Do	5	2.0	2.0			Good
	2	4.0	3.0			Bad	4	2.5	3.0			Bad
0.5%	2	3.0	3.0			Do	4	2.0	2.0			Do
	2	2.0	1.5			Do	2	1.0	0.5			Very bad
	1	2.0	2.0			Do	3	1.0	0.5			Do

TABLE 4.—*Influence of sodium nitrate upon plants inoculated with Lupin bacteria*

Lupin							Serradella					
Concentration	No. of seedlings	Length in average	Average length of root	No. of nodules of 1 plant	Total No. of nodules	Remarks	No. of seedlings	Length in average	Average length of root	No. of nodules of 1 plant	Total No. of nodules	Remarks
Control	2	cm. 4.5	cm. 5.0	3, 3	6	Good	5	cm. 2.0	cm. 2.5	2, 2, 1, 3, 3	11	Good
	2	4.0	3.5	2, 3	5	Do	3	1.5	2.0	4, 2, 2	8	Do
0.005%	2	5.0	4.5	3, 3	6	Do	6	2.5	3.0	3, 2, 2, 3, 4, 1	15	Do
0.01%	2	5.0	5.0	4, 6	10	Best	5	2.5	2.5	3, 4, 3, 2, 3	15	Do
	2	4.5	4.5	6, 1	7	Very good	4	3.0	2.5	3, 5, 2, 2	12	Best
0.02%	2	5.0	5.0	3, 2	5	Do	3	2.5	2.0	9, 8, 4	21	Very good
	2	5.0	5.5	5, 4	9	Best	4	3.0	4.0	3, 5, 5, 5	18	Best
0.05%	2	4.5	5.0	4, 2	6	Do	4	2.7	2.5	3, 4, 2, 3	12	Very good
	2	5.0	5.0	3, 4	7	Very good	5	2.5	3.5	2, 3, 2, 5, 1	13	Do
0.1%	2	Not germinated	5.5	6, 4	10	Best	5	3.0	3.0	1, 2, 5, 7, 1	16	Best
	2	6.0	5.5	3, 3	6	Very good	3	3.5	3.0	2, 3, 5	10	Very good
0.2%	2	5.0	5.0	1, 3	4	Do	3	3.0	3.0	4, 7, 5	16	Best
	2	5.0	5.0	2, 1	3	Good	4	2.5	2.5	3, 6, 2	11	Very good
0.5%	1	4.0	3.5			Good	4	2.0	2.5	4, 2, 2, 1	9	Good
	2	3.0	3.0			Bad	4	2.0	1.5			Bad
1.0%	2	3.0	3.0			Do	5	2.0	1.5			Do
	2	1.5	1.0			Very bad	1	1.0	1.0			Very bad
	2	2.0	1.0			Do			Withered			



TABLE 5.—*Influence of sodium nitrate upon plants inoculated with Serratella bacteria*

Lupin							Serratella					
Concentration	No. of seedlings	Length in average	Average length of root	No. of nodules of 1 plant	Total No. of nodules	Remarks	No. of seedlings	Length in average	Average length of root	No. of nodules of 1 plant	Total No. of nodules	Remarks
Control	2	4.0	3.5	2, 1	3	Good	3	1.8	cm.	3, 1, 4	8	Good
	2	4.0	4.0	3, 2	3	Do	4	2.0	2.5	1, 3, 4, 5	13	Do
0.005%	2	4.5	5.0	2, 3	5	Very good	5	2.5	3.0	3, 2, 5, 4, 2	16	Do
0.01%	2	4.5	4.5	2, 1	3	Good	5	2.0	2.5	2, 3, 1, 1, 4	11	Do
	2	6.0	5.0	2, 4	6	Best	5	3.0	3.5	3, 2, 3, 3, 2	13	Very good
0.02%	2	5.0	4.7	4, 3	7	Do	4	3.5	3.0	4, 2, 3, 4	13	Do
	2	4.5	4.5	2, 1	3	Very good	5	2.5	3.0	3, 5, 5, 2, 1	16	Do
0.05%	1	5.5	5.0	5	5	Best	5	3.0	3.0	2, 3, 2, 1, 2	10	Do
	2	5.0	5.5	2, 4	6	Do	5	3.0	3.5	3, 3, 2, 4, 2	14	Best
0.1%	2	5.5	5.0	1, 3	4	Very good	2	2.5	2.5	5, 4	9	Very good
	2	6.0	5.0	4, 1	5	Best	5	3.5	4.0	3, 4, 2, 3, 5	17	Best
0.2%	2	5.0	4.5	2, 2	4	Very good	5	3.0	3.0	2, 2, 8, 1, 4	17	Do
	2	5.0	4.0	1	1	Good	4	3.0	3.0	4, 2, 3, 2	11	Very good
0.5%	2	5.0	4.0	1, 2	3	Very good	4	2.5	2.5	1, 3, 1, 4	9	Good
	2	3.0	3.0			Bad	4	2.0	2.0			Bad
1.0%	2	1.5	1.0			Do	3	1.0	1.0			Do
	1	2.0	1.0			Do			Withered			

TABLE 6.—*Influence of calcium nitrate upon plants inoculated with Lupin bacteria*

Lupin							Serradella					
Concentration	No. of seedlings	Length in average	Average length of root	No. of nodules of 1 plant	Total No. of nodules	Remarks	No. of seedlings	Length in average	Average length of root	No. of nodules of 1 plant	Total No. of nodules	Remarks
Control	1	5.0	cm.	4	4	Good	5	cm.	cm.	3, 2, 2, 1, 1	9	Good
	2	4.5	5.0	3, 2	5	Do	4	2.5	2.5	3, 2, 2, 1	8	Do
0.005%	2	5.0	5.0	3, 2	5	Do	5	2.5	3.0	4, 3, 2, 1, 2	12	Do
0.01%	2	5.0	4.5	3, 4	7	Very good	5	3.0	2.5	3, 2, 1, 3, 5	14	Do
	2	6.0	5.3	3, 4	7	Best	6	3.0	4.0	4, 3, 6, 4, 4	25	Best
0.02%	2	5.0	5.0	3, 3	6	Do	4	3.0	3.5	4, 3, 2, 1	10	Do
0.05%	2	5.0	4.5	2, 5	7	Very good	2	3.0	3.7	3, 5	8	Very good
	2	6.5	6.0	5, 4	9	Best	4	3.0	3.5	3, 4, 5, 5	17	Best
	1	6.0	6.0	4	4	Do	6	3.0	3.6	2, 3, 4, 3, 2, 3	17	Do
0.1%	2	6.0	6.0	3, 3	6	Do	5	3.5	4.0	4, 4, 3, 4, 2	17	Do
0.2%	2	5.0	5.2	2, 1	3	Very good	5	3.0	3.5	1, 3, 1, 4, 5	14	Very good
	1	4.0	4.0	1, 1?	2	Good	5	3.5	4.5	2?	2?	Do
0.5%	2	4.5	4.0			Do	5	3.0	4.0			Do
	2	3.5	3.0			Bad	5	2.5	3.0			Good
1.0%	2	4.0	3.5			Do	4	2.5	2.5			Bad
	2	3.0	2.5			Very bad	4	2.0	2.5			Do
	1	2.5	1.5			Do	2	1.5	1.0			Very bad

TABLE 7.—*Influence of calcium nitrate upon plants inoculated with Serratella bacteria*

Concentration	Lupin						Serratella					
	No. of seedlings	Length in average	Average length of root	No. of nodules of 1 plant	Total No. of nodules	Remarks	No. of seedlings	Length in average	Average length of root	No. of nodules of 1 plant	Total No. of nodules	Remarks
Control	2	5.0	5.0	1, 2	3	Good	5	2.0	cm.	3, 2, 1, 2, 3	11	Good
0.005%	2	4.5	3.5	2, 2	4	Do	4	2.0	2.0	3, 1, 4, 2	10	Bad
	2	5.0	4.5	2, 3	5	Best	5	3.0	3.0	7, 2, 4, 1, 1	15	Good
0.01%	2	5.0	5.0	5, 2	7	Do	5	2.5	3.0	3, 1, 4, 2, 3	13	Do
0.02%	2	5.0	5.0	3, 2	5	Do	5	3.0	3.0	5, 1, 3, 2, 2	13	Do
0.05%	2	6.5	6.0	5, 3	8	Do	7	4.0	3.5	7, 3, 3, 3, 5, 4, 5	30	Best
	2	6.2	6.0	4, 7	11	Do	5	3.5	3.5	2, 3, 5, 1, 2	12	Do
0.1%	2	6.0	5.5	1, 5	6	Do	5	3.0	3.0	2, 1, 5, 4, 2	14	Very good
0.2%	2	5.5	5.0	3, 4	7	Do	5	4.0	3.0	3, 1, 2, 1, 3	10	Best
	2	5.0	4.5			Very good	5	3.0	3.0			Very good
0.5%	2	4.0	4.0			Do	4	2.5	2.0			Good
	2	4.0	3.0			Good	6	2.5	2.0			Do
1.0%	1	3.5	2.0			Bad	2	2.0	1.5			Bad
	1	2.0	1.0			Very bad	3	2.0	1.0			Very bad
	1		Withered				4	2.0	1.0			Do

TABLE 8.—*Influence of ammonium sulfate upon plants inoculated with Lupin bacteria*

Lupin							Serradella					
Concentration	No. of seedlings	Length in Average	Average length of root	No. of nodules of 1 plant	Total No. of nodules	Remarks	No. of seedlings	Length in average	Average length of root	No. of nodules of 1 plant	Total No. of nodules	Remarks
Control	1	cm. 4.3	cm. 4.0	3, 1	4	Good	5	cm. 1.8	cm. 2.0	3, 1, 1, 2, 1	8	Good
	2	4.0	4.0	2, 3	5	Do	3	1.5	2.0	4, 2, 1	7	Do
0.005%	2	4.5	5.0	2, 3	5	Very good	5	2.0	2.8	2, 1, 3, 3, 2	11	Do
0.01%	1	3.5	4.0	3	3	Good	5	2.5	2.5	3, 4, 1, 5, 1	14	Do
	2	4.5	4.5	3, 3	6	Very good	3	2.5	3.0	2, 3, 3	8	Do
0.02%	1	5.0	5.0	3, 2	5	Do	4	3.0	2.6	2, 3, 3, 1	9	Very good
	2	5.0	4.5	2, 4	6	Do	4	3.0	3.0	1, 3, 5, 1	10	Do
0.05%	2	5.0	4.0	3, 4	7	Do	3	3.0	2.5	2, 1, 3	6	Do
	1	5.0	4.0	3	3	Good	5	2.5	3.0	3, 5, 4, 2, 1	15	Do
0.1%	2	4.5	4.5	1, 3	4	Do	3	3.0	3.2	3, 1, 5	9	Good
	1	3.0	2.0			Bad	3	2.0	2.0			Bad
0.2%	2	3.0	1.5			Do	3	1.5	2.0			Do
	2	3.0	1.5			Very bad	3	1.8	1.5			Very bad
0.5%	2	2.5	1.0			Do	2	1.5	1.5			Do
	1	1.5	1.0			Do	3			Withered		
1.0%	2		Withered			Do	2			Do		
	2		Do			Do	1			Do		
	2		Do			Do	1			Do		

TABLE 9.—*Influence of ammonium sulfate upon plants inoculated with Serratella bacteria*

Concentration	Lupin						Serratella					
	No. of seedlings	Length in average	Average length of root	No. of nodules of 1 plant	Total No. of nodules	Remarks	No. of seedlings	Length in average	Average length of root	No. of nodules of 1 plant	Total No. of nodules	Remarks
Control	2	3.8	3.5	1, 3	4	Good	4	1.8	cm.	1, 2, 5, 1	9	Good
0.005%	2	4.0	3.5	2, 3	5	Do	5	2.0	2.0	1, 3, 1, 3, 2	10	Do
	1	4.0	4.0	2	2	Do	5	2.5	2.6	3, 2, 3, 1, 2	11	Do
0.01%	2	4.5	4.0	3, 3	6	Do	3	2.0	2.3	3, 5, 1	9	Do
	2	4.0	4.5	2, 5	7	Very good	4	2.5	3.0	1, 2, 6, 2	11	Very good
0.02%	2	4.0	4.0	2, 3	5	Good	4	2.5	2.5	1, 3, 5, 2	11	Do
	2	5.0	5.0	4, 1	5	Very good	4	3.0	3.0	2, 2, 1, 5	10	Do
0.05%	2	5.0	4.5	3, 4	7	Do	3	2.5	2.7	3, 1, 2, 2	8	Good
	2	4.5	3.5	5, 1	6	Good	5	3.0	2.5	1, 2, 2, 2, 3	10	Very good
0.1%	2	5.0	4.0	1, 2	3	Do	5	2.0	1.8	1, 1, 2, 3, 3	10	Good
	2	3.5	3.0			Bad	5	2.0	1.5			Bad
0.2%	1	4.0	2.7			Do	5	1.5	1.0			Very bad
	2	3.5	2.5			Very bad	3	1.5	1.0			Do
0.5%	2	3.0	2.0			Do	4	1.0	0.5			Do
	1	3.0	2.0			Do	4	1.0				Do
1.0%	2	2.0	1.5			Do	2	1.0	0.5			Do
	1	Withered					1			Withered		
	2	Do					2			Do		

# STUDIES OF THE NODULE BACTERIA OF GENGE

K. ASŌ AND S. OHKAWARA

*Tokyo Imperial University, Japan*

Genge (*Astragalus sinicus*), known also as Rengeso or Shiunei, is a herbaceous legume which is supposed to be of the origin of southeastern Asia and to have been introduced into Japan in ancient times through China. The herb grows wild abundantly everywhere in this country except in the northern districts. It flowers in spring very flourishingly, sometimes carpeting beautifully in reddish purple the banks and fields with its racemes.

Now-a-days this plant species is cultivated as a cover crop and as a green manure and is highly prized as an economic plant of a distinct value to Japanese agriculture. Genge is quite hardy through winter, being classified as a biennial crop. It is deep-rooted and grows most vigorously in a well drained land. The vines of a matured plant are about 0.3 m. long in case of the early maturing, and from 1 to 1.5 m. in the later maturing varieties. The amount of green matter harvested per acre is about 7.5 to 15.0 tons and sometimes more, and the nitrogen content of the green harvest is about 0.48 per cent and that of the air-dry matter, about 2.25 per cent.

Most soils in Japan appear to be supplied naturally with proper bacteria for the formation of the root-tubercles on Genge. However, it is sometimes necessary to supply the nodule bacteria especially to the soil of newly cultivated fields.

An account is here given of our own studies on the morphology and physiology of the nodule bacteria of Genge since no previous record on the subject is found in the literature so far as we are aware.

The nodule bacteria of *Astragalus* are somewhat smaller in size than those of peas, soybeans and others, varying from  $0.7 \times 1.5$  to  $0.9 \times 1.8 \mu$ . They are motile having some flagella, we observed, stained by Fischer and Löffler's methods, one or two polar flagella actually, but the exact number of flagella remains undetermined. The organisms on soil-extract-mannit-agar form a white, turbid, round, elevated colony with a paraffin lustre. It is interesting to note that they grow on potato medium far better than do the nodule bacteria of other leguminous plants.

On the soil-extract-mannit-agar noted above, the nodule bacteria of *Astragalus* generally do not multiply so well as do other nodule bacteria, but the former grow better upon the addition of a small quantity of

orizandin, a substance containing vitamins. Further they produce a slimy substance which is less slimy than similar substance produced by the nodule bacteria of peas and soybeans.

The optimum pH of culture media for the growth of the *Astragalus* nodule bacteria is between 6.5 and 7.5, the limiting pH (on the acid side) being 4.7. In the course of growth, they also produce some acids which are of a higher acidity than those produced by other strains of nodule bacteria. Finally, they can utilize cane sugar as well as pentoses and hexoses.

# SOME EFFECTS OF STOCK AND SCION RELATIONSHIP UPON THE LEGUME NODULE ORGANISM

T. E. RICHMOND

*Ohio State University, U. S. A.*

## INTRODUCTION

When more reliable information upon the life cycle of the nodule organism has been obtained, and the mechanics of the relation of the host plant to the nodule organism is better understood, then it may be possible to answer the age old question of just why this symbiotic relationship is common only to the legumes. The importance of any information which would help explain this symbiotic relationship might be of inestimable value in working out a similar relationship between the non-legumes and some form of nitrogen assimilating organism. As no fundamental relationship has as yet been reached upon which a satisfactory grouping of the nodule organism for inoculation purposes can be made, the work of some outstanding investigators of the nodule organism will be taken up with the hope that it may be found to be less contradictory than previously supposed.

## HISTORICAL

The discovery by Hellinck and Wilfarth of the symbiotic relationship between the nodule organism and leguminous plants led investigators in various parts of the world to study this relationship. The resulting literature upon the subject is full of contradictory statements. The question whether there is a single species of legume nodule organism or a separate race of bacteria for each kind of plant is still not definitely settled.

It was the opinion of Beijerinck, who first isolated a pure culture of the legume organism, that the nodule forming organisms of the legumes belonged to one species, divided into several groups with slight modifications, to which the name *Bacillus radicicola*, *Beijerinck*, has been given.

As the result of their experiments Nobbe, Hiltner, and Schmid were of the opinion that all the nodule bacteria of the different legumes studied were of a single species, *B. radicicola*, but these bacteria are influenced by the plants in whose roots they live to such a degree that their descendants are able to infect readily only that species of legume to which the host plant belonged. They also believed that neutral nodule bacteria were present in soils which had never grown legumes and that as different



legumes were introduced these neutral nodule bacteria were able to enter the roots of the different legumes and cause nodule formation. Zipfel made use of serological methods for the first time in studying the nodule organism, and as a result of further work along this line by Klimmer, Kruger, and Simon, divided the nodule organism into 9 species differing sharply from one another.

As the result of carefully conducted greenhouse experiments, Burnell and Hansen divided the nodule organisms into 11 groups interchangeable for the purposes of inoculation, with the statement that the adaptations as tested by actual inoculations upon plants are constant. For example, the soybean organism not only retains its individuality as tested by serologic methods and by cultural characteristics, but it also retains its special adaptation for the soybean plant, in spite of imposed conditions designed to break this adaptation. These facts form perhaps a legitimate basis for the belief that distinct species exist among the nodule bacteria. In numerous other characteristics, however, these bacteria are so much alike, and as a whole they differ so widely from any other species of bacteria, that it seems more consistent to regard the adapted forms as varieties of the single species *Ps. radiculicola*.

As the result of extensive investigations Joshi is of the opinion that nodule formation should not be taken as the sole physiological test for distinguishing the species, but that nitrogen-fixation and plant stimulation thereby is the essential function of the nodule organism which he believes to be one single species.

Löhnis, Fred and Hansen report that the nodule bacteria of the leguminous plants are to be divided into two groups, differing morphologically as well as physiologically. The first group shows all features characteristic of *Bacillus radiculicola*, *Beijerinck*, and is found upon legumes of European origin. The second group is characterized by a single polar flagella and is found upon legumes of Asiatic origin.

The results of Shunk confirms the views of the above investigators in dividing the nodule organism into two groups, *Bacillus* and *Pseudomonas radiculicola*.

From the above results the fact that the legume nodule organisms are of two groups, one common to cultivated legumes of Europe, the other of Asia, which differ morphologically as well as physiologically has been established beyond question.

Recent work upon the legume nodule organism has shown that any single natural group may be divided in two or more strains, with inherent differences as to nodule formation and nitrogen-fixation.

Investigators working on the symbiotic relationship of host to organism have for the most part arrived at the conclusion as summarized by Löhnis and Fred that, "the very sharp adaptations of the different types of nodule bacteria to their host plants are probably partly due to the prefer-

ence of the special degree of acidity that is characteristic of the sap of these plants."

That this theory is open to critical study is apparent when one finds that the acidity of the sap of the same legume, grown in soils of different degrees of acidity, may vary more than the differences found between different legumes when grown in a neutral soil.

### REVIEW OF PRELIMINARY WORK

In the Botanical Gazette for December, 1926, I presented the results of preliminary work upon the inoculation of grafted bean plants, a brief review of which follows.

As it is possible to grow to maturity a grafted lima bean (*Phaseolus lunatus*) top on a navy bean (*Phaseolus lunatus*) root, or a navy top on a lima root and as the nodule bacteria of the lima bean are distinct from those of the navy bean an entirely untouched method of investigating the relation of the host plant to its nodule producing organism is opened for study.

In the preliminary work sterilized seeds were planted in quartz sand and allowed to grow to a height of from 6 to 8 inches before washing out and making the desired grafts. Grafting was performed by cutting half the stem away for a distance of about 4 inches. A lima and navy seedling thus treated were placed so that the two cut surfaces were together and bound firmly to each other with string. After the seedlings were securely fastened to each other, the united stem was punctured in several places with a needle. The combined plants were then replanted in quartz sand in a gallon jar and tied to a support. In about a week's time the stem of one of the two beans was nearly cut in two between the root and bottom of the graft, and at the same time the top of the other plant was cut off. In a few days' time the cut root was severed and removed from the jar. This would then leave a lima bean root with a navy bean top, or a navy root with a lima bean top as the graft happened to be made. Grafts were also made so that two tops grew upon a single root or a single top on two roots. The plants were in all cases watered with tap water, which was found to be not only free of the nodule organism, but also contained sufficient plant food elements, with the exception of nitrogen, to grow plants to maturity.

After a successful graft had been made, the nodule organism to be used was added to the jar and the plant allowed to grow for the required time.

The following conclusions were drawn from the preliminary work:

(1) When a lima bean top is growing upon a navy bean root, in quartz sand, a pure culture of lima bean bacteria will not cause the formation of nodules upon the roots of the grafted plant, but the roots are stimulated, the plant grows to maturity, and appears to be able to obtain atmospheric nitrogen.

(2) When such a grafted plant is inoculated with a navy bean culture, nodules are formed and the plant grows to maturity. The navy bean organism in the root nodules apparently are able to furnish nitrogenous compounds to the lima bean top in exchange for carbohydrates synthesized by a top not normal to the nodule organism used.

(3) A lima bean top grafted upon an inoculated navy bean root grew to maturity and developed seeds.

(4) Similar results are obtained with the reciprocal grafts and inoculations.

(5) When seeds are produced by a grafted plant, either lima or navy, they are so modified that plants grown from such seeds no longer have the power of selective adaptation for the specific nodule organism common to it, but are inoculated by either the lima or navy bean organism.

From these preliminary results two lines of investigation have developed:

(1) A study of the behavior of plants produced from seeds grown upon grafted plants.

(2) A study of pure cultures of the nodule organisms found in the nodules of the differently grafted plants.

(1) Starting with seed produced upon grafted plants in 1924, five generations of grafted plants have been grown. In each case the plants being used to make the grafts were grown from seed produced by grafted plants. Whenever these seeds were tested out it was always found that nodules were produced upon the roots of both the lima and navy beans by a pure culture of either the navy bean organism or of the lima bean organism. Nitrogen-fixation was just as active in a lima bean plant inoculated with a navy bean culture, as in a similar plant inoculated with a lima bean culture.

(2) A pure culture of the nodule organism was obtained from the nodules found upon the roots of a modified lima bean plant inoculated by a pure culture of the navy bean organism. It was found that the pure culture so obtained would always give nodule formation when used upon either lima or navy beans which had been grown upon grafted plants. When nodules were formed upon modified lima and navy bean plants by a pure culture of the navy bean organism, it was found that pure cultures made from the lima bean nodules would inoculate normal lima beans very well, but had no effect upon normal navy beans. Pure cultures from the navy bean nodules were found to inoculate navy but not lima beans. This shows that by starting with a pure culture of the navy bean organism and using it upon modified navy and lima beans that nodules are formed upon each, and from these nodules pure cultures can be obtained which will cause nodules to form only upon normal lima or navy beans. By passage through a plant grown from modified seed the navy bean organism

has been transformed into a pure culture of the lima bean organism as we now define such pure cultures. The reciprocal inoculations proved that by plant passage through modified seed a lima bean culture can be changed into a pure navy bean culture.

### SUMMARY

Seeds grown upon grafted plants are so modified that plants grown from such seeds no longer have the power of selective adaptation for the specific nodule organism common to it, but are inoculated by either the lima or navy bean nodule organism.

Pure cultures of the nodule organism found upon the roots of lima or navy bean plants, grown from modified seed, will always cause nodule formation upon either lima or navy bean plants grown from modified seed.

By passing a navy bean culture through a plant grown from a modified lima bean seed, a pure culture of the lima bean organism may be obtained.

By passing a lima bean culture through a plant grown from a modified navy bean seed a pure culture of the navy bean organism may be obtained.

# A COMMON ERROR IN THE METHOD OF TOTAL NITROGEN ESTIMATION IN SOILS AND ITS BEARINGS ON THE RESULTS OF NITROGEN-FIXATION EXPERIMENTS

D. V. BAL

*Department of Agriculture, Central Provinces, India*

## INTRODUCTION

In a previous publication (2) the writer has shown that when the total nitrogen is estimated by the Kjeldahl process on the dry or air-dry sample low results are obtained. It is necessary to moisten the sample before analysis. This was discovered during a course of experiments conducted to find out the extent of nitrogen-fixation in wheat soils from the Jubbulpore district, Central Provinces, India. The figures were not published in detail since on the whole it was found that no measurable nitrogen-fixation took place in those soils under the laboratory conditions employed.

Other writers (4) have, however, since published work on nitrogen-fixation in soils from which it appears that an error may possibly have crept in owing to the fact that certain of their nitrogen estimations were carried out on air-dry soil and subsequent estimations on moist soil showed an apparent increase due to the fact that moist soil yields the whole of its nitrogen by the Kjeldahl process. This apparent increase appears to have been wrongly ascribed to nitrogen-fixation.

It has, therefore, been considered desirable to publish the results obtained by the writer in detail and bring them to the notice of the biological section of the Conference of the International Society of Soil Science since these results, and the method proposed for the estimation of nitrogen in soils, have a direct bearing on the problem of nitrogen-fixation in soils.

## EXPERIMENTAL

Some biological aspects of the wheat cultivation in the Haveli tract, Jubbulpore district, have been discussed in a previous publication (3). In this tract, wheat has been grown continuously for centuries practically without any addition of manure in the form of cattle dung or green manure. The constituents (1) of the soil are, therefore, being removed year after year and it would be of interest to know how far the natural recuperative agencies help to keep up the nitrogen content of those soils and the extent to which cultivation affects this process. Two plots of

land from Kheri Farm, Jubbulpore, were selected for this purpose. One of these was given a shallow cultivation according to the prevailing system of the Haveli tract, described in the previous publication (3), while the other was ploughed deeply every year.

Attention was first directed to determine the extent of nitrogen-fixation in the soil under laboratory conditions without any artificial addition of organic matter. Samples of soil were taken from the plots mentioned above on October 27, 1922, soil from the first and second foot being collected separately. Fifty g. of air-dry soil, passed through a 1 mm.-sieve, was placed in a beaker and the moisture content was made equal to that found in the field at planting time. All experiments were started in duplicate and separate beakers were put up for each determination. Estimations of total nitrogen were made every 2 weeks. For each determination, the whole contents of the beaker were employed, half being used for the estimation of ammonia, nitrite and nitrate; and the other half, for the estimation of organic nitrogen.

This experiment was only a preliminary one and was primarily designed to determine in the first instance the various working difficulties, etc. The estimations of nitrogen on the original sample were made by the usual *dry* method then followed. In the subsequent estimations the same method was employed also during the first 6 weeks. The wet soil, containing about 25 to 32 per cent of water, was directly weighed out in the Kjeldahl's flask, strong sulfuric acid was added and the digestion was carried out as usual. Certain experimental difficulties were experienced however in removing the soil from the beakers to the Kjeldahl flask. In order, therefore, to facilitate this process the soil was carefully washed into the Kjeldahl flask and sulfuric acid added in the usual way. By this procedure a much higher percentage of nitrogen was obtained in the 8th week as is seen in Table 1 (see figures in italics for the 8th week in Table 1). The residue left after the digestion of the soil was also very fine and perfectly white in contradistinction to the coarse and blackish residue given by the usual dry method. The idea, therefore, occurred to the writer that this large difference might be due to the preliminary water treatment of the soil.

In order to determine if such was the case, the nitrogen in the original air-dry sample was also determined afterwards in the same way and it was learned that the increase of nitrogen obtained after 8 weeks was only apparent; the difference being due to the imperfection of the usual Kjeldahl method as applied to the soils analyzed in the dry condition (see figures for the original nitrogen percentage in Table 1).

The *wet* method which was further worked out and published (2) separately thus originated from this preliminary experiment and the results are given in Table 1.

These results generally indicated that there was no measurable change

TABLE 1.—*Preliminary comparison of nitrogen determined by wet and dry method*

Soil treatment	Mois- ture in dry soil	Total nitrogen on dry soil				
		At start	After 2 weeks	After 4 weeks	After 6 weeks	After 8 weeks
Unploughed 1st foot	per cent 25.0	per cent 0.0336 0.0444	per cent 0.0348 0.0355	per cent 0.0273 0.0268	per cent 0.0330 0.0326	per cent 0.0378 0.0462
Do 2nd Do	32.2	0.0344 0.0431	0.0367	0.0215 0.0249	0.0372 0.0438	0.0433
Ploughed 1st Do	27.3	0.0300 0.0413	0.0226	0.0284 0.0273	0.0301 0.0398	0.0400 0.0427
Do 2nd Do	29.9	0.0285 0.0399	0.0243 0.0255	0.0238 0.0242	0.0419 0.0385	0.0408 0.0419

in nitrogen content in any of the soils employed, when incubated for a period of 8 weeks and with a moisture content varying from about 25 to 32 per cent.

Having obtained these results it was decided next to find out if measurable nitrogen-fixation did take place in these soils with varying moisture contents by employing the newly devised *wet* method for the estimation of nitrogen throughout the whole period.

The general plan of this experiment was similar to that employed in the first one, duplicate beakers being used for each determination and no organic matter added, as a source of energy. The results obtained are given in Table 2.

TABLE 2.—*Nitrogen in a mixture of soils from first and second foot of the Haveli system and cultivated unmanured plots determined by wet method*

Moisture in dry soil	Total nitrogen on dry soil				
	At start	After 2 weeks	After 4 weeks	After 6 weeks	After 8 weeks
per cent 7.5	per cent 0.0445	per cent 0.0434	per cent 0.0428 0.0434	per cent 0.0448 0.0461	per cent 0.0433 0.0450
15.0	Do	0.0440 0.0440	0.0413	0.0449 0.0461	0.0439 0.0434
22.0	Do	0.0458 0.0453	0.0452 0.0437	0.0463 0.0475	0.0470
30.0	Do	0.0459 0.0448	0.0431	0.0464 0.0464	0.0461
37.5	Do	0.0461 0.0459	0.0460 0.0456	0.0452 0.0465	0.0454
45.0	Do	0.0455 0.0449	0.0467 0.0462	0.0454 0.0454	0.0473 0.0489

While considering the results given above it is essential in the first instance to have some idea of the experimental error with a view to finding out the significance of these figures. In all these experiments 0.1 *N* acid and alkali were employed for the purpose of titration and the indicator used was methyl orange. It was observed on almost all occasions in duplicate or even quadruplicate estimations, that the normal variation in titration figures was from 0.1 to 0.2 cc. of acid. Now if 25 g. of soil is employed for the determination of nitrogen, the variation in nitrogen content should normally be  $0.2 \times 4 \times 0.0014$  or about 0.00112 per cent nitrogen. Since the original nitrogen in the soil was 0.0445 per cent any figures which are outside  $0.0445 \pm 0.00112$  or, in other words lower than 0.0434 or higher than 0.0456, can be taken as significant.

Considering the figures given in Table 2 on this basis it will be observed that with a low moisture content such as 7.5 or 15 per cent there is no nitrogen-fixation. With a moisture content ranging between 30 and 45—and particularly with 45 per cent—there seems to be some indication of nitrogen-fixation.

From these results it appeared however that a period of 2 months' incubation is perhaps not sufficient to obtain any appreciable nitrogen-fixation.

A third set of experiments was therefore started with fresh soils; the samples being taken on June 20, 1923. In this experiment the moisture content was maintained at 15, 30 and 45 per cent but the period of incubation was increased to 6 months, determinations of nitrogen being made every 2 months. Duplicate beakers were used for each estimation and no organic matter was added, as a source of energy. The figures obtained are given in Table 3.

Taking into consideration the experimental error, in the case of ploughed soil, figures lower than 0.0449 or higher than 0.0471 and in the case of the Haveli soil figures lower than 0.0452 or higher than 0.0474 can be taken as significant. Thus the figures given above show that in the soil from the ploughed area there was no measurable fixation of nitrogen with a moisture content of 15 or 30 per cent but on the other hand there was a tendency towards loss of nitrogen from the soil. With 45 per cent of moisture the original nitrogen content was about maintained.

With Haveli soil also there was a certain amount of loss of original nitrogen when the moisture content was kept at 15 and 30 per cent. With a moisture content of 45 per cent, however, the soil showed a certain amount of nitrogen-fixation instead of a loss at the end of 6 months.

From the results given above it will be seen that on the whole, under laboratory conditions, there does not seem to be much nitrogen-fixation going on in these wheat soils, which have no addition of organic matter. In this connection one practical aspect of the situation, however, deserves careful consideration. Thus it will be seen that with our existing methods



we can not very well determine a difference of 0.001 to 0.002 per cent of nitrogen in the soils. If we consider that an acre of soil to a depth of 8 inches weighs roughly two million pounds it is evident that a fixation of 0.001 to 0.002 per cent of nitrogen would mean an addition of 20 to 40 lb. of nitrogen per acre which is more than enough for the average wheat crop obtained from these soils.

TABLE 3.—*Influence of time upon nitrogen-fixation*

Soil treatment	Moisture on dry soil	Total nitrogen on dry soil			
		At start	After 2 months	After 4 months	After 6 months
	per cent	per cent	per cent	per cent	per cent
Kheri unmanured soil, deeply ploughed	15	0.0460	0.0422	0.0420	0.0417
			0.0427	0.0421	0.0411
	30	Do	0.0396	0.0424	0.0413
			0.0412	0.0424	0.0413
	45	Do	0.0412	0.0445	0.0453
			0.0435	0.0431	0.0475
Kheri unmanured soil, shallow cultivation	15	0.0463	0.0454	0.0454	0.0446
			0.0448		
	30	Do	0.0451	0.0447	0.0446
			0.0445	0.0453	0.0439
	45	Do	0.0459	0.0417	0.0487
			0.0475		0.0497

Finally the writer wishes to emphasize the importance of taking into consideration the important factor of the method of nitrogen determination while formulating any conclusions regarding the work on nitrogen-fixation in soils in general and heavy soils in particular. The writer feels justified in urging the necessity of adopting the *wet* method for the estimation of nitrogen in all soils in routine analytical work in agricultural laboratories.

The *wet* method adopted by the writer is as follows: Twenty to 25 g. of air-dry soil are transferred to a 500 cc. Kjeldahl flask and 50 cc. of distilled water added to it, and the soil is shaken for a short time and allowed to remain for about 30 minutes. The requisite quantity of strong sulfuric acid is now added and the flask heated as usual. Copper sulfate and potassium sulfate are added when white fumes of decomposed sulfuric acid have just begun to be evolved. After the oxidation is completed the distillation is completed in the usual way.

## LITERATURE CITED

- (1) Evans, G. 1913. Agr. Jour. India, 8: 117.
- (2) Bal, D. V. 1925. Jour. Agr. Sci. [England] 15: 454.
- (3) Plymen, F. J., and Bal, D. V. 1920. Agr. Jour. India, 15: 289.
- (4) Sahasrabuddhe, D. L., and Daji, J. A. 1925. Mem. Dept. Agr. India, Chem. Ser. 8: 53.

# THE TRANSFORMATIONS OF NITROGEN IN MANURE

B. NIKLEWSKI

*Poznan University, Poland*

## INTRODUCTION

The activity of nitrogen in the microbiological processes of soil and manure heaps has been the subject of numerous investigations during the last century. It is a problem of great importance to microbiology as well as to practical agriculture.

It has been stated, particularly by Wagner, Schneidewind and Schulze that during a period of 4 years plants absorb hardly more than 40 per cent of the nitrogen contained in manure, in some cases even less than 15 per cent. These fluctuations indicate that in dry well aerated loose soil the absorption exceeds 30 per cent, while on heavier, more compact soil it falls beyond this limit. Schneidewind fixes the amount of nitrogen in manure absorbed in loess soil at 25 per cent during a period of 4 years.

The question is, what becomes of the remaining 75 or in the best of cases 60 per cent of nitrogen? This nitrogen unabsorbed by plants, is transformed into (a) albumin in bacterial bodies and into humus; (b) gets infiltrated into deeper layers of the soil; (c) and divides into free atmospheric nitrogen, susceptible to the influence of denitrifying bacteria.

## THE FIXATION OF NITROGEN BY BACTERIA

The fixation of bacteria is only temporary and does not prevent this nitrogen from passing later into the plant organism, at the relative period, however, it means a loss which can have a deplorable effect on crop yields. The transformation of nitrogen into albumin removes nitrogen from the absorbing influence of plant roots. The result is a starvation of plants. Especially when fresh manure is employed, that starvation is so considerable, that manure does not act at all. Therefore, agricultural practice does not recommend the use of fresh manure. If a farmer employs it, he only grows siliquoses which are not susceptible to nitrogen fertilization.

The explanation of this statement will be found in the following experiment made by Niklewski. Oats have been grown in flower pots and given a surplus of phosphoric acid and potash with a minimum of nitrogen. Light sandy soil was used with a small addition of clay. One portion, 1 N, of nitrogen supplied by sulfate of ammonia amounted to 0.2 g. N = 0.968. 2 N was a double portion 0.4 g. N; 4 N a quadruple one 0.8 N; 6 N was a

six-fold portion 1.2 g. N=5.808 ammonium sulfate. Chopped wheat straw, of 5 mm. length, was added at a rate of 1 S=20 g., 2 S=40 g. of straw per pot.

TABLE 1.—*The influence of straw on oat production*

Nitrogen fertilizer with addition of straw	Derived from 3 flower pots	
	Straw	Grain
	grams	grams
No nitrogen, no straw	32.57	24.04
1N	70.20	54.30
2N	94.90	81.70
1N+1S	35.70	27.47
2N+1S	74.70	56.24
4N+1S	106.40	93.00
1N+2S	22.00	16.16
4N+2S	100.30	82.50
6N+2S	119.60	121.50

Fresh straw considerably diminished the crop which was improved by a sufficient amount of nitrogen. In this case straw exerted no specially detrimental effect upon plant roots; it only reduced nitrogen by decomposition and was injurious, if too little of it was present. As soon as there was plenty of nitrogen at hand a beautiful crop was obtained notwithstanding a large portion of straw, amounting to 40 g. per pot. Two tenths of a gram of N and 20 g. straw were equivalent in their influence upon the development of plants. 2 N+1 S produced the same effect as 1 N without straw. Two tenths of a gram of N constituted nutrition for the bacteria that developed on 20 g. of straw. Quite analogously it may be supposed that fresh manure with a large amount of undecomposed organic substance deprives plants of soluble food. In consequence fresh manure applied shortly before planting can entirely starve plants so that they grow poorer than without manure. But given early, in plenty of time before planting, fresh manure will do no harm if only most of its matter gets decomposed in the soil beforehand. This explains why in inclement climatic conditions in cold grounds, in the mountains or in the North, farmers generally use well fermented manure so that plants in their short period of growth may make use of available nutrients.

The infiltration of nitrogen, contained in stable manure, into soil has not been investigated by the writer.

#### DENITRIFICATION IN SOIL

Very serious losses of nitrogen may result from the fact that nitrification and denitrification processes occur simultaneously, liberating free nitrogen. A mutual interaction of these two processes is only possible when

the organic substance is in contact with the dissolving nitrogen compounds of liquid manure. In this case namely, the nitrogen in liquid manure undergoes nitrification, and nitrates thus produced decompose through the influence of denitrifying bacteria that feed on the organic substance contained in the solid parts of manure. Therefore, if the solid parts of manure are quickly separated in the soil from the liquid, the liberation of free nitrogen is stopped.'

'The fact that light active soils better utilize the nitrogen in manure than do cold damp ones, is due not only to an easier access of oxygen and fuller oxidation of organic substances but it is also a question of quicker or slower separation of solids and liquids of manure. I have not made further experiments on this problem.'

'But there arises the question whether a simultaneous process of nitrification and denitrification is possible. I investigated this matter of nitrogen transformations in manure itself during storage in the dung heap.

#### LOSSES OF NITROGEN IN STABLE MANURE

Researches regarding losses of nitrogen in manure have been carried out for about 70 years, but their results, nevertheless, do not answer the needs of agricultural practice.

Völker<sup>1</sup> states that manure in normal storing loses 30 per cent of nitrogen. Holdefleiss found, in 1884, that during a seven month fermentation, nitrogen in manure diminished 23 per cent. Müntz and Girard demonstrated, in 1892, that the losses of nitrogen in normally stored manure amounted, during a four weeks' period, to from 30 to 55 per cent.

It has been stated that one of the most important causes of nitrogen losses, besides that due to volatilization of ammonia, was the liberation of free atmospheric nitrogen which in turn is due to microbiological processes. In 1894, Jentys, a Polish scientist found that in a closed atmosphere where oxygen had large access, free nitrogen volatilizes from horse manure. He stated this not only on the basis of nitrogen balance in manure, but also by means of analyzing the gas products. Out of 100 g. fresh horse manure, containing 445 mg. nitrogen, at the end of 15 days 19.08 per cent or 84.9 mg. of nitrogen was liberated, and at the end of 33 days 20.74 per cent or 92.3 mg. was obtained.

In order to obtain a more definite statement of nitrogen losses caused by the liberation of free nitrogen Pfeiffer submitted 2 kg. of fresh manure to an energetic aeration in a flask, drawing the liberated gases through sulfuric acid. The amount of ammonia passing from the flask into the acid was minimal. But the total losses of nitrogen from the manure amounted to 42.6 per cent, at the end of the tenth month.

Free nitrogen, therefore, was liberated from manure during normal fermentation.

<sup>1</sup> Jour. Agr. Soc. Eng. 1857.

## WHY FREE NITROGEN WAS LIBERATED FROM MANURE

Two hypotheses were available for the explanation of the process of free nitrogen liberation from stable manure. Namely, some authors supposed that the influence of some unknown bacteria was responsible for the oxidation of ammonia into free nitrogen. Ehrenberg and Reichenbach tried to demonstrate by experiments the impossibility of accepting the second hypothesis, namely of the interaction of nitrification and denitrification. But Niklewski proved the interpretation given by these authors to their experiments, to be erroneous. A second important argument was suggested by Kaserer who apparently isolated a new organism, the *bacillus azotofluorescens*, which decomposed carbon ammonia into nitrogen and formic acid, but it lacks confirmation since until the present there is no further evidence available as to the existence of such an organism. But another supposition by König, and others, proved to be correct and was confirmed by experiments of Niklewski, performed from 1909 to 1923. According to these authors' point of view the liberation of free nitrogen results from the interaction of nitrification and denitrification processes. The strongest arguments against this hypothesis are brought forth by the experiments and conclusions of the Russian scientists Winogradzki and Omelianski, who first succeeded in obtaining pure cultures of bacteria that oxidized ammonia salts into nitrites. Therefore, it was necessary to investigate nitrification conditions in stable manure.

## DOES NITRATE OCCUR IN STABLE MANURE?

I pass over the fact of nitrate arising in centers with plenty of organic compounds; this method of producing nitrate being developed during the sixteenth, seventeenth and eighteenth centuries into a large industry, so as to become the only means of securing nitrate, indispensable to the manufacture of explosives. I only quote the experiment of Holdefleiss who proved that manure which at first contained no nitrate contained considerable amounts of it after being stored on the dung heap for 7 months.

The following figures from an experiment, carried out at Wangern 1884, give the per cent of nitrate in an average manure.

Manure without treatment	Manure covered with soil	Manure with superphosphate	Manure with a potash salt
0.0101	0.108	0.053	0.035

The fact, stated by Holdefleiss, that liquid manure introduced into normally stored manure considerably reduces the amount of salpeter nitrogen, is of special importance to our subject (Wangern 1886). The same author's observation, that stable manure kept under cattle contained very little saltpeter is equally valuable (Sadewitz 1886).

## CONDITIONS FOR DEVELOPMENT OF NITRIFYING BACTERIA IN STABLE MANURE

The fact that we find no trace of nitrates in fresh or in a manure a few weeks old is no proof against the possibility of nitrifying bacteria developing in it. These organisms may exist and produce nitrate which momentarily can be subject to denitrification and thus liberated as free nitrogen.

Comparable experiments performed by Niklewski obtained the following results. Small portions of manure, of different origin, were incubated upon a mineral medium generally used for growing nitrifying bacteria and it was found that bacteria that oxidize ammonia into nitrous acid were present in manure kept in the dung pit. By mixing manure with sterilized water and shaking it well, and by growing small portions of this mixture on mineral medium, the approximate number of active nitrifying bacteria cells in a unity of stable manure could be determined. Their number was found to be 32,000 cells per gram of manure. The experiment being performed during a heavy frost, while the ground was covered with snow, a general contamination of the manure by soil was out of the question. Fresh manure contains very few nitrifying bacteria since there are none in fresh excrements nor in urine; only straw contains some. Small particles of old manure collected on the stable floor proved to be a very prolific source of contamination. In manure that had been lying in the dung pit for 1 or 2 weeks a great many bacteria were found, over 50,000 per gram of substance. Evidently they find highly favorable conditions there for development; whereas old, tightly packed horse manure was entirely void of nitrifying bacteria.

Manure from an underground stable, where it had been kept lying under the feet of the cattle, taken from two different farms and from different spots in the stable, gave quite dissimilar results. By means of diluted inoculations it was stated that no bacteria or hardly any were present. A single test showed some 6,400 bacteria per gram of substance.

These observations prove that nitrifying bacteria do not find such favorable conditions for development in manure kept under the cattle as in manure stored in the dung pit.

Niklewski holds that this difference is not due to the lack of bacteria, which are so very plentiful in nature, nor to the difficulty of aeration although very often in textbooks of agricultural chemistry these circumstances are supposed to provide better conditions for the conservation of manure under the cattle. By littering a great amount of oxygen is introduced into manure and nitrifying bacteria are conspicuous by their ability to utilize a minimum of oxygen. Accordingly in the dung pit nitrifying bacteria were found at a considerable depth; from 50 to 60 cm.

Urine concentration proved to be a decisive factor concerning the development of nitrifying bacteria. On mineral medium a 1:10 solution of

urine, as well as highly diluted fermented urine, showed a toxic influence upon these bacteria.

The influence of fermented urine on nitrification:

Mineral medium plus a solution of urine: 0.5 per cent $(\text{NH}_4)_2\text{SO}_4$ Per cent of urine	0	1	10	20	40	60	80
Diphenylamine reaction, at the end of days	5	7	8	17	0	0	0

The above data demonstrates the toxic influence of urine on nitrifying bacteria, therefore none were found in the basin of fermented urine. An extract of diluted excrements, however, had no injurious effect upon nitrification.

Consequently, it can be admitted that in manure kept under cattle, urine checks the development of nitrifying bacteria whereas, the lack of it in the dung pit creates favorable conditions for their growth.

### THE INFLUENCE OF NITRIFYING BACTERIA UPON THE NITROGEN BALANCE IN STABLE MANURE

Considering the nitrogen losses in stable manure it was necessary to determine whether nitrifying bacteria present produced nitrate that would be split into a *statu nascendi* by denitrifying organisms.

With this object Niklewski used a liquid mineral medium that contained small amounts of stable manure, gathered with a careful exclusion of nitrifying bacteria. Excrements and urine were collected so as not to touch the floor of the stable and straw was sterilized. Proportions in the mixture of excrements, urine and straw were such as commonly occur in stable manure. To 200 cc. of mineral medium containing 0.2 per cent ammonium sulfate were added 10 or 25 g. of the above manure; part of the cultures were inoculated with nitrifying bacteria and part were left uninoculated. At the end of 41 days nitrogen was determined in the cultures with the following results:

TABLE 1.—Action of nitrifying bacteria upon solid and liquid manure

Medium 200 cc. 0.2% $(\text{NH}_4)_2\text{SO}_4$ and	Nitrifying bacteria	N in culture at end of 41 days	Qualitative reaction	
			Nitrates or nitrites	Ammonia
10 g. manure	Uninoculated	mg.	Absent	Present
Do	Inoculated	29	Present	Absent
20 g. manure	Uninoculated	141	Absent	Present
Do	Inoculated	65	Do	Absent

The above results are drawn from a series of similar experiments which gave analogous findings without a single exception. The inoculation of



nitrifying bacteria caused considerable loss of nitrogen. The difference between inoculated and uninoculated cultures amounted from 100 to 29 mg. and 141 to 65 mg. Nitrifying bacteria completely oxidized the ammonia during the experimental period and only nitrogen from excrement and straw remained in the culture; the latter yielded 65 and 29 mg. of nitrogen.

In cultures that were inoculated with nitrifying bacteria complete lack of ammonia was noted, whereas nitrites and nitrates appeared in inoculated cultures only when 10 g. of manure were used while none were present in a 25 g. portion. The explanation of this fact is a 10 g. portion of manure is too small an amount of organic substance to be able to develop enough nitrifying bacteria for reducing all of the oxidized nitrogen compounds while in a 25 g. portion of manure there was a sufficient amount of organic matter for this purpose.

As this experiment was performed on a liquid mineral medium, differing widely from normal circumstances under which stable manure is usually kept, Niklewski carried out an analogous experiment on normally kept manure in 1922 and 1923.

He put per 900 g. of manure, gathered as before, into loosely covered glass dishes after having determined accurately the content of nitrogen in each. Some of the cultures were inoculated with bacteria. At the end of 11 months in a temperature of 28 to 30° C. tests were made which showed the following results:

TABLE 2.—*Effect of bacteria upon manure incubated direct; nitrogen at 11 months*

Nitrifying bacteria	Nitrogen *	Nitrogen	Loss in	Loss expressed as per cent of original N
	mg.	per cent	mg.	
Inoculated	2196	0.323	577	20.8
Do	2120	0.300	653	23.6
Uninoculated	2697	0.377	76	2.7
Do	2690	0.418	83	3.0

\* Nitrogen at the beginning of the experiment, 2773 mg. N.

The small losses in uninoculated cultures are due to volatilization of ammonia in loosely covered dishes. The influence of nitrifying bacteria upon nitrogen losses in inoculated cultures is shown most distinctly.

By means of this experiment it was stated that nitrifying bacteria are largely responsible for nitrogen losses. In uninoculated cultures ammonia and amine nitrogen accumulated as the analyses in Table 3 and 4 proved.

These experiments show that nitrifying bacteria find excellent conditions for development in stable manure and cause serious losses of nitrogen.

TABLE 3.—*Effect of inoculation upon formation of ammonia in manure*

	Expressed as N	Relation to total N	N in manure
	mg.	per cent	per cent
Inoculated	33	1.51	0.0049
Do	15	0.71	0.0021
Uninoculated	180	6.67	0.0253
Do	172	6.39	0.0267

TABLE 4.—*Effect of inoculation upon formation of amine nitrogen in manure*

	Expressed as N	Relation to total N	N in manure
	mg.	per cent	per cent
Inoculated	4	0.18	0.0007
Do	1	0.05	0.0001
Uninoculated	542	19.64	0.0758
Do	442	16.43	0.0688

### NITROGEN LOSSES IN MANURE WHEN KEPT UNDER THE CATTLE AND IN THE DUNG PIT

The above experiments carried out by Niklewski greatly elucidate the matter of the remarkably well performed experiments made by Märker, at Lauchstädt, in 1898.

It has then been proved that the conservation of nitrogen in manure kept in dung pits, one of them being covered with a roof and the other open, was less efficient than in manure that was kept lying under the cattle where losses amounted only to a third of the losses suffered in the dung pit. Namely, these were only 13.3 per cent; whereas in the dung pits the losses reached 36.9 and 37.4 per cent.

The cause of this fact must be attributed, on the basis of the above experiments made by Niklewski, to the fact that in manure kept under the cattle urine constantly thrown out by the animals checked the development of nitrifying bacteria and, therefore, nitrogen was sufficiently conserved, and losses consisted mostly of volatilized ammonia. Nevertheless, the nitrification process in manure can not be said to be entirely checked when kept under the cattle, it occurs also in the front part of the stable, but is reduced. Whereas in the dung pit nitrifying bacteria find excellent conditions for development, and, therefore, losses are considerable, as the soluble and adaptive nitrogen vanished almost entirely.

The favorable influence of covering manure with soil, other than raising its temperature, and by loose storing as is practised in Germany, is probably due to the checking of nitrifying bacteria in their growth.

At any rate, the development of nitrifying bacteria ought to be considered in further investigations on conservation of nitrogen in stable manure.

# NITROGEN AVAILABILITY IN FUNGUS AND BACTERIAL CELLS FOR NITRIFICATION AND CELLULOSE DECOMPOSITION IN THE SOIL

C. BARTHEL AND N. BENGTESSON  
*Central Institute, Sweden*

## INTRODUCTION

It is evidently of great interest, from a soil-biological point of view, to get a deeper knowledge concerning the processes by which the proteins of the dead cells of the soil microorganisms again enter into the nitrogen cycle and thus become available for plant life.

In order to obtain a satisfactory answer to this question it is necessary, of course, to have pure preparations of the microorganisms which are to be examined for the purpose mentioned above.

The investigations concerning the nitrification of bacteria and mould substance in the soil, published by Bierema (1), are not very valuable from an experimental standpoint, and for that reason we shall not discuss them here.

Our own researches on this subject were made both with bacteria and mould substance.

## NITRIFICATION OF MOULD SUBSTANCE

The mould substance used in these experiments was produced by cultivating *Aspergillus niger* in *Raulins* solution. The cultures were grown in large, flat vessels, covered with glass plates and held in the incubator at 25° C. The culture medium as well as the vessels and the glass plates were, of course, sterilized. After 12 days a thick, coherent surface vegetation was formed, which was washed in distilled water several times, pressed by hand and ground in a sausage machine. One hundred grams of fungus substance were obtained in this way; 50 g. were then autoclaved during 5 minutes at 120° and the other 50 g. was left unsterilized.

The two portions were analyzed for moisture, ammonia nitrogen and total nitrogen, with the following result:

TABLE 1.—*Analysis of unsterilized and sterilized mould*

	Unsterilized mould	Sterilized mould
	per cent	per cent
Total N	0.88	0.91
Ammonia N	0.02	0.02
Dry substance	24.91	21.83

The two samples of mould substance were carefully mixed with a good clay soil of neutral reaction. Portions of 1 kg. were kept in glass jars plugged by paraffined corks, perforated by small glass tubes containing cotton plugs.

Two parallel jars of each kind were prepared after the following schedule:

Jar No.	Contained
I a and b	1 kg. of soil
II Do	Do + 10 g. unsterilized mould
III Do	Do + 10 g. sterilized mould

The moisture content of the soil was adjusted to 18 per cent and the jars were kept in an incubator at 18 to 20° C. during 4 months. Fortnightly, during the first 2 months, and once a month, during the last two months the samples were analyzed for dry substance, nitrate nitrogen and pH. It would take too much space to communicate all these figures and, therefore, we will confine ourselves to the results recorded in Table 2.

TABLE 2.—Analysis of soil and mould inoculations

Jar No.	Nitrate N per kg soil	Mould-N nitrified		pH
		mg.	Per cent	
I a and b	39.5			6.7
II Do	87.6	48.1	54.7	6.7
III Do	76.8	37.3	41.0	6.7

The figures are average numbers from the two parallel jars and represent the results after 3 months, when the maximum of nitrification was reached.

During the experiment there was a vigorous growth of fungi in the jars with unsterilized and sterilized *Aspergillus* substance (but not in the control jars). It is possible that the nitrification of the mould nitrogen in reality has been still higher, but that the nitrate formed was partly reduced by the growing mould (4).

The sterilized mould shows a poorer nitrification than does the unsterilized, which fact probably depends on the effect of the high temperature on the mould protein.

## NITRIFICATION OF BACTERIAL SUBSTANCE

The investigations concerning bacterial substance were started in order to get information on two questions, namely the process of nitrification of the bacterial cells in itself, and further the possibility for the cellulose fermenters in the soil to use the cell substance as a source of nitrogen by the decomposition of added cellulose.

The bacterial preparations used in these experiments were pure cultures of *Urobacillus Pasteurii*, *Bacterium radiculicola* and *Azotobacter chroococcum*, grown in 10-l. cultures, in specially appropriated media solutions. The cultures were constantly aerated during the time of growing. After the maximum of growth was reached, the bacterial mass was separated from the culture solution by means of a centrifuge especially constructed for this purpose. After repeated washings and centrifugations the bacterial substance was dried by treatment with alcohol. In this way 3 to 4 g. of bacterial substance was obtained from each culture as a greyish powder. Microscopic examinations showed that the bacteria were mainly unchanged with regard to the form of the cells, only that a part of the *Azotobacter* cells seemed a little shrunken and that the preparations contained only bacterial cells, without any admixture of foreign matter.

The amount of nitrogen was determined in the different preparations, with the following result.

	per cent
<i>Urobacillus Pasteurii</i>	11.41
<i>Bact. radiculicola</i>	3.90
<i>Azotobacter chroococcum</i>	1.63

The nitrogen content of *Urobacillus* being quiet normal, it seems, on the other hand, to be very low for the other two preparations. However, there are in the bacteriological literature statements, according to which the nitrogen content of *Azotobacter* may vary from 1.33 to 12.8 per cent (2, 3) depending upon the amount of slime in the cultures. The same thing may be true for *Bact. radiculicola*.

The nitrification experiments were made in a very poor sandy soil, whose moisture content was adjusted to 10 per cent, which is the optimum for biological processes in such soil.

Glass jars containing 200 g. of soil, provided with the cork stoppers already described, were prepared, some of which received different amounts of ammonium sulfate; others, 0.2 g. of different bacterial preparations. Check samples without any addition were also prepared. The jars were kept in an incubator at 18 to 20° C. The results of these experiments are recorded in Table 3, where the amount of nitrate nitrogen in the control jars (7.0 and 6.1 mg. per kg. soil after 2 and 4 months respectively) is subtracted.

As shown in Table 3, the nitrification of the ammonium sulfate has proceeded rapidly with the smaller amount of this salt. Thus, the nitrifying power of the soil was quite normal. With the larger quantity of ammonium sulfate the nitrification process is going on much slower, which circumstance is explained by the fact that the pH in this poorly buffered soil sunk from 6.7 to 4.0 after 4 months in the jars with 24 mg. of ammonium sulfate nitrogen.

With regard to the different bacterial preparations, it is very surprising that the *Azotobacter* preparation does not nitrify at all. Of *Bact. radiculicola* 34.1 per cent is nitrified and of *Urobacillus* 60.7 per cent after 2 months. After two months more the nitrification has not proceeded beyond these amounts. It is not unlikely that a part of the nitrate nitrogen formed has been used by the soil microorganisms for the breaking down of the carbonaceous matter contained in the bacterial cells.

TABLE 3.—Nitrification experiments with poor, sandy soil

Added N in mg. per 200 g. of soil	Amount of N nitrified	
	After 2 months	After 4 months
	per cent	per cent
12 Ammonium sulfate	33.8	84.3
24 Do	20.3	32.5
3.3 <i>Azotobacter</i>	0.0	0.0
7.8 <i>Bact. radiculicola</i>	34.1	33.8
22.8 <i>Urobacillus</i>	60.7	64.9

We then find that, except for *Azotobacter*, an important part of the nitrogen in the bacterial preparations has been rapidly nitrified under the prevailing conditions.

#### CELLULOSE DECOMPOSITION IN SOIL IN THE PRESENCE OF BACTERIAL SUBSTANCE AS A SOURCE OF NITROGEN

At the same time as the nitrification experiment we also started a cellulose decomposing experiment in order to find out whether the bacterial preparations could be used by the cellulose fermenters as a source of nitrogen. The same sandy soil, as well as the same technic which was used in the nitrification experiments, was also employed for the cellulose investigation. For the determination of the cellulose in the soil, the method of Charpentier, modified by Barthel and Bengtsson was used. The cellulose was added to the soil as ground filter paper, to an amount of 1 per cent of the weight of the soil. The quantities of ammonium sulfate and bacterial preparations were the same as in the foregoing experiment. The results of the cellulose decomposition experiments are recorded in Table 4.

The figures of Table 4 prove clearly that the nitrogen in the bacterial preparations is used just as well as the ammonium sulfate nitrogen by the cellulose fermenters. The amount of cellulose fermented is only dependent upon the amount of nitrogen present, with the exception of the jars with 24 mg. ammonium sulfate nitrogen.

TABLE 4.—*Cellulose decomposition in sandy soil*

Added N in mg. per 200 g. of soil	Cellulose fermented	
	After 2 months	After 4 months
	per cent	per cent
Checks	0.32	10.81
12 Ammonium sulfate	47.00	68.84
24 Do	56.10	96.47
3.3 Azotobacter	0.00	32.23
7.8 <i>Radicicola</i>	29.44	47.75
22.8 <i>Urobacillus</i>	88.22	100.00

## LITERATURE CITED

- (1) Bierema. 1909. Centbl. Bakt. 11. Abt. 23: 716.
- (2) Gerlach and Vogel. 1902-10. Ibid. Abt. 9: 884.
- (3) Hoffman, C., and Hammer, B. W. 1910. Ibid. Abt. 28: 137.
- (4) Koytytchew, S., and Tswetkova, E. 1920. Hoppe-Seyler's Ztschr. Physiol. Chem. 117: 171.

# NITROGEN TRANSFORMATION IN THE DECOMPOSITION OF NATURAL ORGANIC MATERIALS AT DIFFERENT STAGES OF GROWTH

S. A. WAKSMAN AND F. G. TENNEY

*New Jersey Agricultural Experiment Station, U. S. A.*

To be able to understand the reasons for the rapidity of liberation of nitrogen from the decomposition of plants at different stages of growth, we must know the composition of the plant at these various stages and nature of decomposition of the various plant constituents. Although the plant continues to assimilate nutrients, including nitrogen until maturity, the percentage of nitrogen in the plant reaches a maximum at an early stage, then gradually diminishes, reaching a minimum at maturity or a little before maturity. This is true not only of nitrogen but also of certain other elements.

Plant materials decompose more rapidly and the nitrogen is liberated more readily (in the form of ammonia) at an early stage of growth and less so when the plant is matured. Two causes are to be considered here: (1) the rapidity of decomposition of the various plant constituents; (2) the relation of the nitrogen to the carbon content of the plant tissues.

At an early stage of growth, the plant is rich in water-soluble constituents, in protein and is low in lignins. When the plant approaches maturity, the amount of the first diminishes and of the second increases. The water-soluble constituents, the proteins and even the pentosans and celluloses decompose very rapidly provided sufficient nitrogen and minerals are available for the microorganisms. The lignins do not decompose at all in a brief period of time of 1 or 2 months. More so, their presence has even an injurious effect upon the decomposition of the celluloses with which they are combined chemically or physically. The larger the lignin content of the plant the slower does the plant decompose even when there is present sufficient nitrogen and minerals.

It has been shown repeatedly that the organisms (fungi and bacteria) decomposing the celluloses and pentosans require a very definite amount of nitrogen for the synthesis of their protoplasm. Since the cell substance of living and dead protoplasm always contain a definite, although varying, amount of nitrogen and since there is a more or less definite ratio between the amount of cellulose decomposed and cell substance synthesized, depending of course upon the nature of the organisms and environmental conditions, the ratio between the cellulose decomposed



and nitrogen required by the organisms is also definite. This nitrogen is transformed from an inorganic into an organic form. Of course in normal soil, in the presence of the complex cell population, the cell substance soon decomposes, a part of the nitrogen is again liberated as ammonia and a part remains in the soil and is resistant to rapid decomposition. The amount of nitrogen which becomes available in the soil is a balance between the nitrogen liberated from the decomposition of the plant materials and that absorbed by the microorganisms which decompose the non-nitrogenous and nitrogenous constituents. The younger the plant, the higher is its nitrogen content and the more rapidly does it decompose, therefore the greater is the amount of nitrogen that becomes available. The lower the nitrogen content of the plant the less of it is liberated and the more of it is assimilated by microorganisms.

These phenomena can be brought out most clearly when the same plant is examined at different stages of growth. The rye plant was selected for this purpose. The seeds were planted in the fall. The samples taken on April 28 (I), May 17 (II), June 2 (III), and June 30 (IV). In the third sampling the plants were divided into (a) heads, (b) stems and leaves. The fourth sample was divided into: (a) heads, (b) stems and leaves, (c) roots. The plants were analyzed and the rapidity of their decomposition determined, using sand or soil as a medium and 2 g. of the organic matter. In the case of sand some inorganic nitrogen and minerals were added and a soil suspension used for inoculation. The evolution of carbon dioxide and accumulation of ammonia and nitrate nitrogen was used as an index of decomposition. Tables 1 and 2 show the composition of the plant and the amount of nitrogen made available after 26 days of decomposition.

TABLE 1.—Composition of rye straw at different stages of growth on dry basis

No. of sample	Moisture content at time of harvesting	Ash	Nitrogen	Cold water soluble fraction	Pentosans	Cellulose	Lignin
	per cent	per cent	per cent	per cent	per cent	per cent	per cent
I	80.0	7.3	2.39	32.6	15.9	17.2	9.9
II	78.8	5.7	1.76	22.0	20.5	26.1	13.5
III a	57.4	4.9	1.01	18.2	22.7	30.6	19.0
III b	60.2	5.9	2.20	20.3	22.7	20.1	16.0
IV a	15.0	3.2	1.22	4.7	11.9	4.6	13.4
IV b	15.0	3.7	0.22	9.5	21.7	34.6	18.8
IV c	?	?	0.55	4.7	26.6	37.7	21.0

When a plant material contains about 1.7 per cent nitrogen, as in the rye of the second sampling, there seems to be sufficient nitrogen for the

TABLE 2.—*Decomposition of rye at different stages of growth*  
(2 g. of dry material added to 100 g. of sand or soil medium)

Date of sampling	No. of sample	Nitrogen content of material	CO <sub>2</sub> given off in 27 days		Available nitrogen (NH <sub>3</sub> - N - NO <sub>3</sub> - N) absorbed (—) or liberated (—)	
			Sand medium	Soil medium		
		per cent	mg. C	mg. C	Sand medium mg. N	Soil medium mg. N
April 28	I	2.39	337.7	286.8	+10.1	+22.2
May 17	II	1.76	280.5	280.4	+0.8	+3.0
June 3	III a	1.01	215.7	199.5	-12.1	-7.5
Do 3	III b	2.20	261.9	244.8	+5.7	+7.5
June 30	IV a	1.22	269.9	273.7	-4.4	-2.1
Do 30	IV b	0.22	221.4	187.9	-16.0	-8.9
Do 30	IV c <sup>a</sup>	0.55	187.0	158.4	-8.1	-9.4

<sup>a</sup> Root material used in the decomposition was equivalent to 1.67 g. of moisture free and ash-free organic matter.

growth of microorganisms which decompose this material more or less completely. When the plant material contains less than 1.7 per cent of nitrogen, as in the case of the stems and leaves of the third preparation, additional nitrogen will be required, before the organic matter is completely decomposed (speaking, of course relatively, since if a long enough period of time is allowed for the decomposition, less additional nitrogen will be needed). If the organic material contains more than 1.7 per cent nitrogen, as in the case of the plants in the first planting and the heads of the third sampling, a part of the nitrogen will be liberated as ammonia, in the decomposition processes. The difference between the nitrogen content of the heads and this hypothetical figure = 0.5 (2.2 - 1.7) per cent or 10 mg. nitrogen for the 2 g. of organic matter; actually 5.7 mg. and 7.5 mg. of nitrogen were liberated as ammonia in the sand and soil media respectively. The difference between the hypothetical figure and the nitrogen content of the stems and leaves was 0.69 (1.7 - 1.01) per cent or 13.8 mg. nitrogen for the 2 g. of plant material used. Actually 12.1 and 7.5 mg. of nitrogen were consumed in the sand and soil media. Had the decomposition been allowed to proceed further, the results would have approached from both directions the hypothetical figure, and, with prolonged decomposition (of synthesized substances) would have exceeded it.

The decomposition of 10 g. dry portions of the second sampling and 20 g. dry portions of the stems and leaves of the fourth sampling was studied separately in a sand medium containing available nitrogen and minerals. Only the data for the organic matter portion, insoluble in ether and water are reported. The results show that the pentosans and celluloses are rapidly decomposed, while the lignins are affected only

to a very inconsiderable extent. The nitrogen figures are of direct interest here. Just about as much insoluble protein was left in the first as in the second experiment: in the first the protein is considerably reduced; in the second, increased. This tends to explain the activities of the microorganisms in the soil.

The results show that since there is a very definite ratio between the energy and nitrogen consumption of the microorganisms decomposing the organic matter, it is easy to calculate, given a certain amount of plant material and knowing its nitrogen content, whether nitrogen will be liberated in an available form or additional nitrogen will be required within a given period of time. Calculations can also be made as to how much of this nitrogen is required for the decomposition of the plant material and how long it may take before the nitrogen is again made available.

*TABLE 3.—Composition of organic matter at beginning and end of decomposition  
(Sample II)*

Organic matter (free from ether and water-soluble substances and ash)	At beginning of experiment	At end of experiment
	mg.	mg.
	7,465	2,015
Pentosan	2,050	380
Cellulose (calculated)	2,610	610
Lignin	1,180	750
Protein (insoluble in water)	816	253
Unaccounted for	8.6 per cent	1 per cent

*TABLE 4.—Composition of organic matter at beginning and end of decomposition  
(Sample IV, stems and leaves)*

Organic matter present (free from ether and water-soluble substances and ash)	At beginning of experiment	At end of experiment
	mg.	mg.
	15,114	8,770
Pentosan	3,928	1,553
Cellulose	6,262	2,766
Lignin	3,403	3,019
Protein	181	519
Unaccounted for	10.25 per cent	10.41 per cent

# THE INFLUENCE OF SOLUBLE SALTS AND ORGANIC MANURES ON SOIL NITROGEN<sup>1</sup>

J. E. GREAVES

*Utah Agricultural Experiment Station, U. S. A.*

## INTRODUCTION

Many soils of the arid west contain in the surface foot sufficient potassium for the production of maximum crops for from five hundred to fifteen hundred years, sufficient phosphorus for from one hundred to five hundred years and sufficient nitrogen for only thirty to fifty years. Moreover, the second, third, and subsequent feet are equally rich in phosphorus and potassium, whereas the greater portion of the nitrogen is in the surface-foot section. Therefore, it is evident that nitrogen is the limiting factor of crop production in these soils. Where intensive systems of agriculture are in vogue, it may be possible to use nitrogen-carrying commercial fertilizers, but on the extensive areas grown to grain, hay, and root crops, from an economic view point, this is not practical. Consequently, the nitrogen for these crops must be obtained from the air by biological means. The quantities which may be obtained from the atmosphere by legumes under appropriate plant, soil, and moisture conditions can be rather accurately calculated, but the quantity which may be expected from the non-symbiotic nitrogen-gathering bacteria is not so well understood. For these reasons considerable attention has been given to this problem during recent years.

Many of the soils of the arid west have a rich active nitrogen-fixing microflora which is due in no small measure to their composition. They are high in calcium and magnesium carbonate, and contain a good supply of phosphorus and potassium but have a low nitrogen content. They are poor in organic carbon; hence, their native supply of energy is limited. Moreover, there is a marked difference in the quantity of the soluble salts which occur in the various soils. Laboratory tests have shown these soils to vary greatly in nitrogen-fixing powers; consequently, the question arises: Cannot optimum moisture, aeration, salt content, and source of energy be discovered for the nitrogen-fixing microflora of these soils and the results thus obtained be applied to field condition with a resulting appreciable increase in nitrogen? In an attempt to answer this question, extensive work has been done on representative arid soils under laboratory, vegetation-house, and field conditions. It is the province of this article to consider briefly some of the results obtained in this study.

<sup>1</sup> Approved for publication by Director, Utah Agr. Expt. Sta., April 18, 1927.

## INFLUENCE OF SOLUBLE SALTS UPON NITRIFICATION

Extensive laboratory determinations made upon a great variety of soils, varying in physical composition from the very fine loam to the coarse sand, and from the extremely poor to the very fertile soils, gave an optimum moisture content for maximum nitrogen-fixation of approximately 70 per cent of the water-holding capacity of the soil, as measured by the Hilgard method (2). Quantities greater or less than this reduced the nitrogen fixed in the soil. Either 40 or 100 per cent saturation reduced nitrogen-fixation to one-third that occurring at 70 per cent saturation. Now, using 70 per cent of the water-holding capacity of the soil as optimum a study was made of the effect of various salts upon soil gains in nitrogen. From these it was learned that the nitrogen-fixing microflora of the soil is much more resistant to soluble salts than are other beneficial microorganisms which the soil may contain (2). It was further learned that the addition of definite quantities of the chlorides, nitrates, sulfates, and carbonates of sodium, potassium, calcium, magnesium, manganese, and iron to these soils modified their nitrogen-fixing powers. There was usually a stimulation, the degree varying with the salt, the concentration, and the medium in which it was used.

TABLE 1.—Percentages of nitrogen fixed in soil to which various salts were added, the untreated soil being taken as 100 per cent

	Nitrate	Sulfate	Carbonate	Chloride	Average
Sodium	102.7	104.4	107.3	102.6	104.2
Calcium	109.9	102.6	103.3	100.1	104.0
Iron	102.0	100.4	101.8	104.3	102.1
Magnesium	101.0	101.7	101.9	102.2	101.7
Manganese	102.2	102.4	100.0	102.3	101.7
Potassium	101.3	105.5	100.0	100.0	101.7
Average	103.2	102.8	102.4	101.9	102.6

All except three salts stimulated nitrogen-fixation, but there is a wide variation, depending upon the specific salt. The cations arranged in descending order of efficiency are  $\text{Na} > \text{Ca} > \text{Fe} > \text{Mg} > \text{Mn} > \text{K}$ . It is interesting to note that the non-essential element, sodium, heads the list, whereas the essential element, potassium, is at the bottom; hence increased activity cannot be due to the addition of essential nutrients. These soils are extremely rich in calcium and magnesium, yet the addition of salts of these elements to the soil stimulates nitrogen-fixation. Arranging the anions in descending order of efficiency, we have  $\text{NO}_3 > \text{SO}_4 > \text{CO}_3 > \text{Cl}$ . Here is a close correlation between stimulation and plant nutrients, as these soils are deficient in both nitrogen and sulfur. These results are

averages of a greater number of determinations obtained by the tumbler method under laboratory conditions and can be taken only as indicative of what may be expected under natural field conditions. \*

More direct information is available in results obtained on soils kept in 2-gallon jars under vegetation-house conditions. The work was conducted on four soils: (1) The college farm soil, which is the same soil as that used in the above reported work. It was made unproductive by the addition of sodium carbonate, sodium sulfate, and sodium chloride, individually and in various combinations. (2) A natural-occurring alkali soil in which the predominating salts were chlorides. (3) A natural-occurring alkali soil in which the predominating salts were sulfates. (4) A natural-occurring alkali soil in which the predominating salts were soluble carbonates. These soils were placed in 2-gallon jars provided with under-drainage. There were four jars of each of the native alkali soils and of each of the artificially produced alkali soils. One-half of these were leached until an analysis of the drain water indicated that the greater portion of the soluble salts which could be removed by water had been carried from the soil. At the close of the leaching period, three crops—one of crimson clover and two of barley—were grown on the soil, after which the nitrogen-fixing powers of the soil from each pot were determined by inoculating definite quantities of each soil into Ashby solutions, incubating 21 days, and then determining the total nitrogen. The average results are given in Table 2.

These soils had contained the quantity of sodium chloride, sodium sulfate, and sodium carbonate indicated in the table, yet they fixed nitrogen when seeded into appropriate Ashby solutions, thus substantiating the conclusions previously reached by the tumbler method (3) that nitrogen-fixing organisms are much more resistant to soil alkalies than are other beneficial bacteria.

The leaching of the normal soil and the leaching of sodium-sulfate-treated soil increased their nitrogen-fixing powers. This was probably due to an increased *Azotobacter* growth, as characteristic *Azotobacter* films occurred in the Ashby solutions which had been inoculated with leached and non-leached normal soil, leached soil which had contained sodium sulfate, and the natural-occurring sodium sulfate alkali soil. In fact, the membrane formed earlier and was darker in the presence of sodium sulfate than in its absence. The *Azotobacter* was recovered from the solutions producing typical membranes but so far we have failed to recover *Azotobacter* from the other soils. This raises the question: What organisms are fixing nitrogen in these alkali soils? Work is in progress, the object of which is to answer this question.

Determinations were made of the nitrogen content of each pot at the beginning and at the close of the experiment. The quantity of nitrogen added or removed in the crop and water was rather accurately known;

consequently, it is possible to calculate the gain or loss of nitrogen from each soil during the period of the experiment. When this was done, we found appreciable gains of nitrogen had occurred in many of the soils.

TABLE 2.—*Milligrams of nitrogen fixed in 100 cc. of Ashby solution in 21 days (inoculated with 1 g. of soil from the variously treated soils)*

Treatment of soil	N fixed	Excess by leaching
	mg.	mg.
Soil untreated	8.38	
Soil leached	10.03	1.65
Soil plus 2% NaCl	4.67	
Do leached	8.12	3.45
Do Na <sub>2</sub> SO <sub>4</sub>	6.39	
Do leached	9.61	3.22
Do Na <sub>2</sub> CO <sub>3</sub>	1.11	
Do leached	3.36	2.25
Do 1% each NaCl, Na <sub>2</sub> SO <sub>4</sub>	5.51	
Do leached	6.15	0.64
Do Na <sub>2</sub> CO <sub>3</sub>	5.51	
Do leached	7.33	1.82
Do Na <sub>2</sub> SO <sub>4</sub> , Na <sub>2</sub> CO <sub>3</sub>	5.83	
Do leached	8.49	2.66
Do 0.66% each NaCl, Na <sub>2</sub> SO <sub>4</sub> , Na <sub>2</sub> CO <sub>3</sub>	6.07	
Do leached	6.21	0.14
Sodium chloride alkali soil	1.40	
Do leached	1.42	0.02
Sodium sulfate alkali soil	4.62	
Do leached	4.39	-0.23
Sodium carbonate alkali soil	0.51	
Do leached	3.76	3.25

The normal soil leached and unleached lost considerable nitrogen during the period of the experiment. This was also true of the soil which contained 2 per cent of sodium carbonate. Two of the leached soils also lost nitrogen; those which had contained 2 per cent of sodium chloride and those which had contained a mixture of sodium sulfate and sodium carbonate. All the other potted soils, both leached and unleached, made appreciable gains in nitrogen. The increase varied from 39 lb. per acre-foot of soil (in the one containing a mixture of sodium chloride and sodium sulfate) to 1142 lb. in the leached native sodium sulfate containing soil. It is evident from these results that the so-called alkali salts stimulate nitrogen-fixation in soil. It is greatest in the case of sulfates and least in the case of carbonates. The combination of sodium chloride, sodium sulfate, and sodium carbonate is quite effective in increasing nitrogen-fixation in soil. Although the results are irregular, the increases are so great that they must be attributed to gains from the

atmosphere, due primarily to the non-symbiotic nitrogen-fixing micro-organisms. There is the possibility of slight gains having come from the symbiotic nitrogen fixers which were associated with the crimson clover, but roots, stems and leaves in so far as possible were removed from the soil; consequently, the gain from this source must have been small.

TABLE 3.—Pounds per acre of total nitrogen, gained or lost, from alkali treated soils during 2 years

Normal soil		—512
Do	leached	—165
Soil plus 2% NaCl		256
Do	leached	—140
Do	Na <sub>2</sub> SO <sub>4</sub>	288
Do	leached	97
Do	Na <sub>2</sub> CO <sub>3</sub>	—752
Do	leached	35
Do	1% each NaCl, Na <sub>2</sub> SO <sub>4</sub>	29
Do	leached	112
Do	Na <sub>2</sub> CO <sub>3</sub>	313
Do	leached	137
Do	Na <sub>2</sub> SO <sub>4</sub> , Na <sub>2</sub> CO <sub>3</sub>	169
Do	leached	—381
Do	0.66% each NaCl, Na <sub>2</sub> SO <sub>4</sub> , Na <sub>2</sub> CO <sub>3</sub>	327
Do	leached	670
Sodium chloride alkali soil	natural	335
Do	leached	770
Sodium sulfate alkali soil	natural	298
Do	leached	1142
Sodium carbonate alkali soil	natural	54
Do	leached	418

## INFLUENCE OF ORGANIC MANURES UPON NITRIFICATION

These soils are low in organic carbon; consequently, the energy available to the non-symbiotic nitrogen-fixers is limited, and one would expect that as this limiting factor in nitrogen-fixation is removed that there would be greater gains in soil nitrogen. Both field and laboratory findings bear out this expectation. Limited surveys of these soils show that when continuously cropped to wheat that there is an increase in their nitrogen-fixing powers. This is sufficient to add approximately 30 lb. per acre annually to the soil, and (3) is probably due to the supply of energy furnished the soil microorganisms by the plant residues. Adjoining constantly fallow soils were not found to make this gain. For these reasons, considerable work has been done in an attempt to increase nitrogen-fixation in these soils by the supplying of a source of energy to the nitrogen gathering bacteria and also to measure under vegetation-house and field conditions the resulting gain. In the vegetation-house work, soils with optimum moisture content and varying kinds and quan-



tities of plant residues were kept in 2-gallon jars and the nitrogen gains determined. The soils were kept bare and received varying quantities of alfalfa, pea-vines, and straw. The nitrogen content of the soil was determined at the beginning, and after 3 years. The various plant residues were applied on two separate occasions—at the beginning of the experiment and after two years; consequently, during the experiment, each soil received twice the quantity of plant residues given in the table. The average results obtained on duplicate pots are given in Table 4.

TABLE 4.—Pounds per acre of nitrogen added and recovered from soil receiving various quantities and kinds of plant residues

Treatment	Nitrogen added	Nitrogen recovered	Average yearly gain of nitrogen	Total loss or gain from air	Average gain per ton of plant residue
	lb. per acre	lb. per acre	lb. per acre	lb. per acre	lb. per acre
None		106	35	106	
1 Ton alfalfa	100	583	161	483	242
2 Tons alfalfa	200	1111	304	911	228
3 Do	300	668	123	368	61
4 Do	400	1161	254	761	95
5 Do	500	630	43	130	13
1 Ton peas	80	844	248	744	372
2 Tons peas	160	268	36	108	27
3 Do	240	340	33	100	17
4 Do	320	176		-144	
5 Do	400	243		-157	
1 Ton straw	20	252	77	232	116
2 Tons straw	40	252	70	212	53
3 Do	60	-139		-199	
4 Do	80	38		-42	
5 Do	100	-377		-477	

The average yearly gain per acre of nitrogen for the untreated soil was 35 lb. The application of one ton of alfalfa hay on the two different occasions caused a similar soil to gain 161 lb. of nitrogen yearly. Two ton applications caused a yearly gain of 304 lb. of nitrogen per acre. Four and 5 ton applications were less effective, but even these caused considerable gains of nitrogen. Where 1 and 2 ton applications of alfalfa were made to the soil, there were gains of over 200 lb. of nitrogen in addition to that carried to the soil in the alfalfa. Where larger quantities were applied the gain was less than 100 lb. of nitrogen per ton of alfalfa applied to the soil. These results were obtained in duplicate pots and represent 3 years' averages; consequently, they are too few and extend

over too short a period to state exactly the nitrogen gains which may be expected from the use of alfalfa to increase the nitrogen of the soil due to non-symbiotic nitrogen-fixers. It is evident that with these soils and under these conditions small applications of alfalfa cause considerable nitrogen gains due to non-symbiotic nitrogen-fixers. This is from two to four times as great as the nitrogen applied to the soil in the legume. The results where the pea-vines were used as the manure are not so uniform as those obtained with the alfalfa, but it is evident that small applications of pea-vines cause appreciable gains in soil nitrogen, larger applications cause a loss. One and 2 ton applications of straw resulted in an annual increase of approximately 75 lb. per acre. This is slightly over twice that gained by the untreated soil. Larger applications cause a loss in soil nitrogen. Two conclusions may be drawn from these results: (1) The application of organic manures to these soils increases, to a very appreciable extent, their nitrogen-fixing powers. (2) This is greater where the legumes are used than where the non-legumes are the source of the energy.

These results were obtained under vegetation-house experiments and may be different under field conditions. At least three modifying factors are evident: (1) The soil temperature was more nearly optimum over longer periods of time under vegetation-house conditions than under field conditions. This would tend to make the reported results too high. (2) The moisture content was kept optimum throughout the year. (3) They were obtained on fallow soil, whereas under field conditions there is usually a growing crop. The rapid removal of the nitrogen from the soil by the crop growing should stimulate the bacteria to more active fixation.

The various fallow plats which are in permanent fertility tests at the Central Experiment Station (Greenville) at Logan, offer an excellent opportunity for checking these results with field conditions. These plats have been kept fallow since the beginning of the experiment in 1912 and have received varying quantities of barnyard manure and irrigation water. The irrigation water and manure applied yearly to the soil varied from none to 40 inches and from none to 15 tons per acre, respectively. Various combinations of water and manure in the proportions stated were applied to the different plots. Samples were taken at the end of 11 years from all plats to a depth of 3 feet and analyzed for nitrogen-fixing powers and total nitrogen (5). The summarized results for nitrogen-fixation and representing from 12 to 18 determinations made on similarly treated plats are given in Table 5.

The unmanured soil invariably lost nitrogen when incubated with 2 per cent lactose. This was true of each foot-section but was greater in the second and third feet than in the first. Where 5 tons of manure to the acre were applied to this soil for 11 years it was changed from a soil which lost nitrogen on incubation to one which was fixing appreciable

quantities of nitrogen. The fixation was greatest in the first foot but was perceptible even to the third foot-section.

The addition of 15 tons of manure to the acre yearly increased the fixation over that occurring in the soil receiving a yearly application of 5 tons to the acre. However, the actual gain per ton of manure is greater with the 5 ton application than with the 15 ton application. This is the same as the results obtained in 1916, when a bacteriological study was made to the same soil (4).

TABLE 5.—*Milligrams of nitrogen fixed in 100 g. of soil containing 2 per cent of lactose taken from fallow plats receiving quantities of water and manure*

Treatment	N fixed in various foot-sections			Average
	1	2	3	
	mg.	mg.	mg.	
Unmanured	-1.5	-2.7	-2.0	-2.1
5 Tons manure	3.8	2.1	0.2	2.0
15 Do	5.5	5.4	3.1	4.7
No water	0.3	0.1	0.7	0.4
5 In. water	3.5	0.5	0.2	1.4
10 Do	1.2	3.2	1.4	1.9
20 Do	2.6	3.3	1.5	2.5
30 Do	5.3	1.9	0.0	2.4
40 Do	5.9	-0.6	0.7	2.0

The benefit exerted by manure upon the nitrogen-fixing powers of this soil is probably three-fold: (1) The manure carries to the soil a rich nitrogen-fixing and mineralizing microflora. These produce increased quantities of acids which in turn liberate phosphorus which is so essential to the rapid metabolism of *Azotobacter*. (2) The plant residues contained in the manure when acted on by the rich cellulose microflora of this soil become a valuable source of energy to the nitrogen-fixing. (3) The addition of the manure to this soil changed its physical structure, thus increasing greatly the aeration occurring within it. This would retard the growth of the anaerobic nitrogen fixers which are extremely wasteful of energy and would greatly accelerate the activity of the aerobic nitrogen fixers which use much more economically the available energy.

Total nitrogen determinations were also made to obtain an idea of the loss or gain in total nitrogen by this soil due to the application of irrigation water and manure. The average results are given in Table 6. These represent the average of from 12 to 18 determinations made on different plots but receiving the same amount of water or manuring treatment.

During the period of eleven years the soil receiving 5 tons to the acre yearly of manure had received 884 lb. of total nitrogen. It had

TABLE 6.—Total pounds of nitrogen found in one acre-foot (3,600,000 lb. of soil) after receiving varying quantities of water and manure

Treatment	Nitrogen per acre-foot in various foot-sections			Total
	1	2	3	
	lb.	lb.	lb.	lb.
Unmanured	3758	3226	2218	9202
5 Tons manure	4310	3444	2818	10,572
15 Do	4702	3627	3121	11,450
No water	4338	3408	3055	10,800
5 In. water	4274	3444	2827	10,545
10 Do	4274	3524	2647	10,445
20 Do	4444	3469	3726	11,639
30 Do	4199	3389	2511	10,099
40 Do	4078	3359	2507	9944

gained during this time 1370 lb. or 486 lb., more than had been applied in the manure. When considered in the light of the beneficial effect found for small quantities of manure on nitrogen-fixation, it is very likely that most of this gain is due to the non-symbiotic nitrogen fixing-microflora of this soil. The gain came as 552 lb. in the first foot-section, 218 in the second, and 600 in the third foot-section.

The soil receiving 15 tons of manure to the acre yearly had received 2653 lb. of nitrogen. It had gained 2248 lb. of total nitrogen. This is 405 lb. less than was actually applied to the soil in the manure. The first foot-section had gained 944 lb. of nitrogen, the second 401 lb. and the third foot-section 803 lb.

It is quite possible that considerable of the nitrogen which is unaccounted for has been carried from the surface soil by the irrigation water and is not lost due to denitrification. Consequently, the conclusion appears justified that the application of barnyard manure and plant residues to these soils very appreciably increases their nitrogen-fixing powers, and by such means the total nitrogen of the soil may be appreciably increased.

#### LITERATURE CITED

- (1) Greaves, J. E. 1914. A Study of the bacterial activities of virgin and cultivated soils. *Centrbl. Bakt. Abt.* 11. Bd. 41: 444.
- (2) ———, and Carter, E. G. 1920. Influence of moisture on the bacterial activities of the soil. *Soil Sci.* 10: 361.
- (3) ———. 1922. Influence of salts on bacterial activities of soil. *Bot. Gaz.* 70: 161.
- (4) ———, and Carter, E. G. 1916. Influence of barnyard manure and water upon the bacterial activities of the soil. *Jour. Agr. Research* [U. S.]. 6: 889.
- (5) ———, and Nelson, D. H. 1923. The influence of nitrogen in soil in azofication. *Utah Agr. Expt. Sta. Bul.* 185.

# SOIL MICROBIAL STIMULANTS<sup>1</sup>

J. E. GREAVES

*Utah Agricultural Experiment Station, U. S. A.*

## INTRODUCTION

Various substances not classed as plant nutrients when applied to a soil increase its productivity. This may result from a direct or an indirect action of the substance upon the plant. It may stimulate directly the metabolic activity of the plant. Or more often there may be an exchange between the added substance or some element which it contains and insoluble plant nutrients of the soil, there resulting an increase in the quantity of available plant-food. Probably more often it is due to some action on the microflora which in turn increase the total or available nutrients within feeding area of the plants. The total nitrogen may be increased, and the availability of the phosphorus, potassium, sulfur, and iron may be changed.

It has been found that the number of microorganisms in a soil which will develop on synthetic agar is materially changed in a productive calcareous loam by the application of arsenic (7, 11), many soluble salts (6, 13, 12), organic manure (9, 14), and by the mere leaching of the soil (8). The application of from 100 to 200 p.p.m. of arsenic in the form of sodium arsenite to soil was found to increase the bacteria which would develop upon synthetic agar. This increase was from 10 to 800 per cent, depending upon the specific soil and the quantity of arsenic applied to it. The increase was greatest in a highly calcareous silt loam and occurred when 200 p.p.m. of arsenic in the form of sodium arsenite was applied to it. The sodium chloride, sodium sulfate, and sodium carbonate when applied to a soil individually or in various combinations also greatly increased the bacteria of the soil as determined by the plate method. When the salt or salts were applied in sufficient quantities to render the soil barren, the bacterial numbers were greatly reduced, but after the soluble portion of the salt was leached from the soil there was found to be a material increase in microorganisms. A stimulation was also observed when non-alkali productive soil was leached for some time with water. The increase in bacterial numbers resulting from these treatments was often as great or even greater than when similar soil received barnyard manure.

<sup>1</sup> Approved for publication by Director, Utah Agr. Expt. Sta., 23 April 1927.

## EXPERIMENTAL

Average results obtained with a number of different soils are given in Table 1. The salt, arsenic, or manure was allowed to stand in contact with the soil for some time after which counts were made in the ordinary manner.

TABLE 1.—*Colonies developing on synthetic agar seeded with soil variously treated*

Sodium arsenite	Sodium chloride	Sodium sulfate	Sodium carbonate	Manure (tons per acre)		Leached soil
				5	15	
thousands	thousands	thousands	thousands	thousands	thousands	thousands
Untreated 1953	2708	2708	2708	6428	6428	2708
Treated 20,530	3740	2890	8400	9256	13,380	3274
Percentage increase 951	38	7	210	44	108	21

The counts were made on the same day on both the treated and untreated soil. All conditions with the exception of soil treatment were as nearly as possible the same. The determinations with the different treatments were often made at different times, and the results in some cases are averages obtained on different soils; but the treated and untreated in each series were always determined under comparable conditions. This accounts for the wide variation in the untreated soils, of the different series. Whereas the extent of the stimulation varied in different tests and with different soils, yet it appeared in all samples tested. The order of stimulation due to the tested substances on the soils used arranged in decreasing magnitude are sodium arsenite, sodium carbonate, 15 tons of manure, 5 tons of manure, sodium chloride, leaching, and sodium sulfate.

It is remarkable to find that both sodium arsenite and sodium carbonate cause greater increases in the bacterial content of these soils as determined by the plate method than does barnyard manure. Of the compounds here listed those most toxic in higher concentration are the greatest stimulants in low concentration. The soil which had been treated with sodium carbonate and then leached was in very poor physical condition. Moreover, much of the organic carbon and large quantities of the nitrogen had been removed. The readily available phosphorus had been rendered soluble and leached from the soil; consequently, both physical and chemical conditions appear to be less favorable for bacterial multiplication after treatment than before, yet the bacterial numbers as revealed by the plate method are greatly increased by treatment. The most plausible explanation appears to be that there is within the soil some biological factor which normally preys upon the bacteria and limits the number. This is removed or rendered inactive by the various treatments, consequently the bacteria multiply unhindered.

The same treatments increase ammonification, as may be seen from Table 2.

TABLE 2.—Average quantity of ammonia found in soil receiving various treatments  
(Given as milligrams in 100 g. of soil)

Treatment	Sodium arsenite	Na <sub>2</sub> SO <sub>4</sub> soil	Na <sub>2</sub> CO <sub>3</sub> soil	5 Tons manure	15 Tons manure	Leached
Untreated	92	82	82	56	56	82
Treated	105	89	88	79	107	92
Percentage increase	14	8	7	41	91	12

Sodium arsenite was toxic in all concentrates tested, from 1 part to 200 p.p.m. in soils low in organic manure, but when applied to heavily manured soil in the proportion of 20 parts of arsenic in the form of sodium arsenite the ammonia accumulation was increased 14 per cent. Soil which had received 2 per cent of sodium chloride and then leached constantly contained less ammonia than normal soil; but when minute quantities of sodium chloride, and especially ferric chloride were applied to the soil there was an increase in the ammonia. The sulfate and the carbonate stimulate to the same extent and are less effective than the arsenic, leaching, and manure. These results differ from the counts in that the increase is greatest with the manure. However, it must be remembered that the manure is carrying to the soil a supply of readily ammonifiable proteinaceous material, the very constituents in which these soils are low. We have no data on the extent to which these soils are stimulated by inorganic salts after manure has been added; possibly it may be even higher than in its absence. The arsenite stimulated in the presence of manure, but not in its absence. It would appear that there is something which is removed by washing or by arsenic and some soluble salts which is limiting the production of ammonia in these soils. It also manifests itself by a change in the accumulation of nitrates within the soil. The nitrate content of the variously treated soils was determined after they had been incubated in tumblers for 21 days with 2 per cent of dried blood.

Sodium arsenite was found to be toxic in all the concentrations tested from 0 to 200 p.p.m. of the arsenic in the form of sodium arsenite. However, when 85 p.p.m. of arsenic in the form of sodium arsenate was added, there resulted a 60 per cent increase in the soil nitrates. With lead arsenate the greatest stimulation occurred with 40 p.p.m. of arsenic in the form of lead arsenate. There was no increase in nitrification in any of the soils which had received 2 per cent of sodium chloride, sodium sulfate, or sodium carbonate and then leached. In fact, the nitrate

TABLE 3.—Average milligrams of nitric nitrogen found in variously treated soil after 21 days' incubation at 28° C.

	85 p.p.m. Arsenic as sodium arsenate	40 p.p.m. Arsenic as lead arsenate	NaCl soil	Na <sub>2</sub> SO <sub>4</sub> soil	Na <sub>2</sub> CO <sub>3</sub> soil	5 tons ma- nure	15 tons manure	Leach- ing
Treated	10	11	50	50	50	7	7	50
Untreated	16	19	41	48	3	8	33	54
Percentage increase	60	73	-18	-4	-94	14	371	8

production had been materially decreased in each case. This is due to two factors: (1) Sufficient "alkali salts" remained in the soil even after leaching to render it toxic to the nitrifying bacteria. It is certain that the nitrifying bacteria are much more susceptible to the action of soluble salts than are the other beneficial bacteria which have been tested (2). The salt-treated soil after leaching was in such a poor physical state that anaerobic conditions existed within it; consequently, the aerobic nitrifiers have been suppressed. That this is the explanation and not that the nitrifiers are never stimulated by "alkali salts" is certain from the following: Sodium chloride when added to this soil in the concentration of 600 p.p.m. increased nitrification 42 per cent, and when 0.66 per cent each of sodium chloride, sodium sulfate, and sodium carbonate was added to the soil and then leached, nitrification was increased 33 per cent. No stimulation was found when sodium carbonate or sodium sulfate were used singly.

The mere leaching of the fertile soil increased nitrification within it 8 per cent, whereas 5 tons of manure per acre increased it 14 per cent. As would be expected from the low nitrogen content of the soil, nitrification was greatly increased by 15 tons of manure per acre annually.

Nitrogen-fixation was also increased when the variously treated soil was seeded into Ashby solution or when incubated directly with an appropriate carbohydrate.

TABLE 4.—Milligrams of nitrogen fixed during 21 days in 100 g. of soil or in 100 cc. of Ashby solution seeded with soil variously treated

	20 p.p.m. Arsenic as sodium arsenate	200 p.p.m. Arsenic as as lead arsenate	Na <sub>2</sub> SO <sub>4</sub> soil	5 Tons manure	15 Tons manure	Leaching
Untreated	18	16	8.4	9.9	9.9	8.4
Treated	22	22	9.6	10.2	10.9	10.2
Percentage increase	22	37	14	3	10	19



Neither sodium arsenite nor lead arsenate stimulated nitrogen-fixation when placed in the Ashby solution, but when small quantities were added directly to the soil and then the arsenic-treated soil incubated, there was a marked gain in nitrogen over the non-arsenic-treated soil. The application of sodium arsenate, sodium sulfate, and leaching all increased nitrogen-fixation to a greater extent than did the application of barnyard manure.

\* Small quantities of various salts of sodium, potassium, calcium, magnesium, manganese, and iron all increased the bacterial activities of these soils. As a general rule it was found that those substances which are most toxic in a higher concentration were the greatest stimulants in small quantities. In many cases the stimulation was short-lived, that is, the application of the various substances to the soil caused an increased bacterial activity for a short time and then the specific activity dropped to a lower level than it possessed before the stimulant was applied. However, the stimulation was not always short-lived, as in some cases where the soils were analyzed at the beginning of an experiment and at the end of two years there was found to be very appreciable gains in nitrogen.\*

This is shown in Table 5 where the nitrogen found at the beginning and end of the test is reported.

TABLE 5.—Nitrogen gains in soil receiving various treatments after 2 years

Treatment	Gain in nitrogen (lb. per acre)
Normal soil	-512
Leached	-165
2 per cent NaCl in soil	256
2 per cent Na <sub>2</sub> SO <sub>4</sub> in soil	288
2 per cent Na <sub>2</sub> CO <sub>3</sub> , then leached	35
1 per cent each NaCl, Na <sub>2</sub> SO <sub>4</sub>	29
1 per cent each NaCl, Na <sub>2</sub> CO <sub>3</sub>	313
1 per cent each Na <sub>2</sub> SO <sub>4</sub> , Na <sub>2</sub> CO <sub>3</sub>	169
0.66 per cent each NaCl, Na <sub>2</sub> SO <sub>4</sub> , Na <sub>2</sub> CO <sub>3</sub>	327

\* The gain in nitrogen was greatest in the presence of the three salts—sodium chloride, sodium sulfate, and sodium carbonate—and was next greatest in the presence of sodium chloride and sodium sulfate. The soil receiving only sodium sulfate gained 288 lb. of nitrogen per acre-foot of soil. In Ashby solutions seeded with soil which had received sulfates there formed characteristic *Azotobacter* films, and it was possible to obtain *Azotobacter* from these solutions. This was not always the case with other treatments.

## DISCUSSION

The question now arises: Why this increased bacterial activity which has been so uniformly noted in all the bacterial processes and resulting as regularly from a non-plant nutrient as from a plant nutrient. It has been shown elsewhere (10) that it may be due in part to an increase in the available plant-food of the soil resulting from the added substance. This will not explain the entire phenomenon.

\* It is evident that the soil contains something which is limiting the number and activities of the bacteria within the soil. This limiting factor is removed by leaching, arsenic, and many soluble salts. It does not appear probable that it is protozoa as they would not be leached from the soil more readily than bacteria. If it be a toxin it must be soluble in water and inactivated by heat and the various chemical compounds which have been applied to this soil. Can it be a bacteriophage? This has been found in soil (4), both garden and field, but not from prairie and forest soils. It has also been found on the roots and stalks of plants (5). D'Herelle (3) reports that calcium chloride interferes with the bacteriophage of *Shiga bacilli*; that potassium chloride retards the process; and that some salts, when low in concentration, stimulate. However, Brutsaert (1) found that at least some strains of the bacteriophage will withstand 14.5 per cent salt solution. It would be destroyed by heat (2) and would be more likely to occur in soil containing organic manures. Stimulation is considerably more pronounced in soil containing organic manures and is prevented by heating the soil (8). Consequently, it appears at least feasible that the observed phenomenon may be due to a bacteriophagic substance ultramicroscopic in size and having the characteristics of either a virus or a ferment. This occurring in the soil may keep the bacterial activities at a certain level. It is sensitive to heat and chemicals, and is removed by leaching; hence, such treatments of soil would remove or inactivate it, thus permitting the bacteria to multiply unhindered. Work is now in progress, the purpose of which is to test this theory.

## LITERATURE CITED

- (1) Brutsaert, P. 1924. Les Bacteriophages es et les microbes dans le bouillon by persale. *Compt. Rend. Soc. Biol.* [Paris] 90: 646.
- (2) Davidson, W. 1922. Filterable "substance" antagonistic to dysentery and other organisms. *Abstracts of Bacteriology*, 6: 159.
- (3) D'Herelle, F. D. 1926. *The Bacteriophage and Its Behavior*. Translated by G. H. Smith. Williams and Wilkins, Baltimore.
- (4) Dumas, J. 1920. Sur la prisence du bacteriophage l'intestin sain, dans l'terre, et dans l'eau. *Compt. Rend. Soc. Biol.* [Paris] 83: 1314.
- (5) Gerresten, F. C., et al. 1923. Das Vorkommen eines Bakteriophagen in den Wurzelknollchen der Leguminosen. *Centbl. Bakt (etc.)*, Abt. 11:60, 311.

- (6) Greaves, J. E. 1916. The influence of salts on the bacterial activities of the soil. *Soil Sci.* 2: 443.
- (7) ———. 1916. Stimulating influence of arsenic upon the nitrogen-fixing organisms of the soil. *Jour. Agr. Research*, [U. S.] 6: 389.
- (8) ———. 1927. The microflora and productivity of leached and non-leached alkali soil. *Soil Sci.*, 23: 271.
- (9) ———, and Carter, E. G. 1916. Influence of barnyard manure and water upon the bacterial activities of the soil. *Jour. Agr. Research*, [U. S.] 6: 389.
- (10) ———, ———. 1919. The action of some common soil amendments. *Soil Sci.* 7: 121.
- (11) ———, ———. 1924. Influence of sodium arsenite on microflora of soil. *Bot. Gaz.*, 77: 63.
- (12) ———, ———, and Goldthorpe, H. C. Influence of salts on the nitric nitrogen accumulation of the soil. *Jour. Agr. Research* [U. S.] 16: 107.
- (13) ———, ———, and Lund, Y. 1922. Influence of salts on azofication in soil. *Soil Sci.* 13: 481.
- (14) ———, and Nelson, D. H. 1923. The influence of nitrogen in soil on azofication. *Utah Agr. Exp. Sta. Bul.* 185.

# SOME NOTES ON THE CONDITIONS OF NITRIFICATION

J. HENDRICK

*University of Aberdeen, Scotland .*

## INTRODUCTION

It is often stated even in standard authorities that nitrification is checked or prevented by an acid medium, for instance, in such a well known Manual as "Soil Conditions and Plant Growth" by Sir John Russell, 5th Edition, 1927, p. 258, it is stated, "The Organisms (of nitrification) will not tolerate an acid medium; a sufficient excess of calcium carbonate is therefore necessary both in culture solutions and in soils." On the other hand it is sometimes recognized that acidity does not necessarily prevent nitrification. Professors Lyon and Buckman in their well known work on "The Nature and Properties of the Soil" 1922, state, "It has generally been considered that nitrification was very much retarded if not actually brought to a standstill in an acid soil. Recent data, however, seem to indicate that the process will proceed in acid soil, although the addition of lime in some form is usually beneficial." It appears, therefore, to be necessary to obtain further evidence as to the degree of tolerance which the organisms which cause nitrification exhibit towards acid conditions. Some drainage experiments which we recently carried out in the Soil Department of the University of Aberdeen, furnish some evidence on this point, although they were originally designed with another object in view. They also give some evidence on the effect of ordinary plant nutrients in stimulating the rate of nitrification and indicate that a supply of calcium carbonate is unnecessary even when nitrification takes place at a very rapid rate.

Some account of these experiments has already been published (1). The experiments were carried out in small artificial drainage tanks 10 inches in diameter and 20 inches deep, filled in their natural order with soil and subsoil from the Experimental Farm at Craibstone, Aberdeen. This is a granitic soil containing large reserves of partially weathered feldspars and other compound silicates. It is also well supplied naturally with both humus and phosphate. Accounts of the composition of this soil have already been published (2). This soil represents a type which occurs extensively in the north of Scotland. It is naturally of considerable fertility but is generally more or less acid in reaction and is apt to be shallow and to suffer readily from drought. The soil used in these exper-

iments had to begin with, a pH value of about 5.7, and was therefore distinctly acid. The experiments were intended to throw light on the effect on the drainage of the addition of manurial substances in heavy dressings and also upon the power of such a soil to fix and retain the constituents of manures. Tank 1 was unmanured, Tank 2 was manured with sulfate of ammonia only, Tank 3 with sulfate of ammonia and superphosphate and Tank 4 with sulfate of ammonia, superphosphate and muriate of potash. The manures were mixed into the few inches of surface soil and afterwards distilled water was applied with a sprinkling apparatus in quantities to represent a heavy rainfall and the drainage water which came through was collected.

The tanks were manured four times at intervals of a few months the amount of manure applied being gradually increased. At the first manuring the manures were applied at the following rates:

Tank 2, sulfate of ammonia 5 Cwt. per acre.

Tank 3, superphosphate 10 Cwt. per acre.

Tank 4, Muriate of potash 5 Cwt. per acre.

All materials, both the manures and the substances recovered in the drainage have been calculated into rates per acre. At the first manuring each of the Tanks, 2, 3 and 4, received only one kind of manure, at subsequent manurings Tank 2 received sulfate of ammonia only, while Tank 3 received both sulfate of ammonia and superphosphate and Tank 4 the same manures and muriate of potash in addition. The period which elapsed between the first and second manurings is referred to as Period 1, that between the second and third manurings as Period 3 and that after the fourth manuring as Period 4.

## EXPERIMENTAL

After the first manuring drainage was collected until it was found that all the chloride applied as muriate of potash to Tank 4 had been recovered. A certain amount of chloride was also found in the drainage of the other tanks. As shown in Table 1, this amount was very similar in the case of Tanks 1, 2 and 3, the amount recovered from Tank 4 however is almost exactly equal to the amount applied as muriate of potash plus the amounts recovered from the other tanks which received no muriate. The composition of the drainage water of the first period, expressed in pounds per acre, is shown in Table 1.

During the second period sulfate of ammonia was applied to Tank 2 at the rate of 10 cwts. per acre, or double the amount given in the first dressing. Tanks 3 and 4 received sulfate of ammonia at the same rate together with superphosphate at the rate of 20 cwts. per acre, and in addition Tank 4 received muriate of potash at the rate of 10 cwts. per acre. At the third manuring each tank again received the same dressing

TABLE 1.—Materials applied and recovered from drainage tank experiments  
(Pounds per acre)

Tank	First Period						
	1	2		3		4	
	Unmanured	Sulfate of ammonia		Superphosphate		Muriate of potash	
	Recovered	Applied	Recovered	Applied	Recovered	Applied	Recovered
Nitrogen as ammonia	0.75	119	0.75		0.5		0.75
Do nitrate	122.25		245.5		133.25		142.75
Phosphoric acid	1		1	160.25	1		1
Sulfuric acid	67	339	321	359.75	340.25	1.5	109.5
Chlorine	41.25		42	1	42	273	317
Lime	167		415.25	304	301	1	345
Magnesia	41.5		200.5	9.75	89.5	1.5	84.75
Potash	10.25		14		10	272	13.25
Soda	102.25		136		175.5		152
Silica	57.5		104		116		265.25

as at the second manuring. At the fourth manuring the quantities were again doubled and Tanks 2, 3 and 4 received sulfate of ammonia at the rate of 20 cwts. per acre. Tanks 3 and 4 superphosphate at the rate of 40 cwts. per acre and Tank 4 muriate of potash at the rate of 20 cwts. per acre. Table 2 shows the results so far as ammonia and nitrate are concerned.

TABLE 2.—Nitrogen applied as manure and recovered in drainage water from drainage tank experiments  
(Pounds per acre)

Tank	1	2		3		4	
	Unmanured	Sulfate of ammonia		Sulfate of ammonia and superphosphate		Sulfate of ammonia, superphosphate and muriate of potash	
	Recovered	Applied	Recovered	Applied	Recovered	Applied	Recovered
1st Period N as ammonia	0.75	119	0.75	0	0.5	0	0.75
N as nitrate	122.5		245.5		133.25		142.75
2nd Do N as ammonia		237.25		237.25		237.25	0.25
N as nitrate	56		248.75		236.75		275.25
3rd Do N as ammonia	0.25	237.25	0.25	237.25	1	237.25	7.5
N as nitrate	65.25		340.75		307		340.25
Total of periods N as ammonia	1	593.5	1	474.5	1.5	474.5	8.5
N as nitrate	244		835		677		758.25
4th Period N as ammonia	0.25	474.5	1.25	474.5	3.5	474.5	13.25
N as nitrate	193		774		792		776
Total of 4 periods N as ammonia	1.25	1068	2.25	949	5	949	21.75
N as nitrate	437		1609		1469		1534.25

The results given in Table 1 show that when sulfate of ammonia was applied to Tank 2 at the rate of 5 cwt. per acre, or 119 lb. of nitrogen per acre, there was no increase in the amount of nitrogen recovered as ammonia in the drainage water as compared either with the unmanured

Tank 1 or with the Tanks 2 and 3 which were manured with superphosphate and muriate of potash respectively. Any nitrogen recovered as ammonia from any of the tanks was in mere traces which could only be determined in the drainage water by nesslerization. On the other hand all the nitrogen applied as sulfate of ammonia to Tank 2 was recovered as nitrate in the drainage water. The recovery is almost quantitative, for if we add the amount of nitrogen recovered from the unmanured Tank 1, 122.25 lb. to the amount of nitrogen applied as sulfate of ammonia to Tank 2, 119 lb. the result is 141.25 lb. whereas the amount actually recovered as nitrate was 245.5 lb. It is to be noticed that the amounts of nitrogen recovered from Tanks 3 and 4 was distinctly greater than that recovered from the unmanured Tank 1. So far as it goes this indicates that the application of superphosphate to Tank 3 and of muriate of potash to Tank 4 increased the amount of nitrate naturally formed in these soils. This means presumably that it increased first the decomposition of organic matter in the soil with consequent production of ammonia and subsequently the production of nitrate from that ammonia. This point is referred to further below. Table 1 also shows that chlorine added to Tank 4 as potassium chloride was quantitatively recovered in the drainage water but that on the other hand the potash was almost completely retained by the soil. Sulfate whether applied as sulfate of ammonia or in superphosphate is largely recovered in the drainage water. It is also noticeable that the amount of sulfate recovered from Tank 4, to which practically no sulfate was applied, is very considerably greater than that recovered from Tank 1. This again indicates increased bacterial activity in the soil as the result of manuring with muriate of potash, for the increased amount of sulfate was probably derived from the organic sulfur of the humus.

The bases, lime, magnesia and soda are all recovered in increased amounts from Tanks 2, 3 and 4 as compared with Tank 1. In the case of Tank 2 the increase is accounted for by the nitric and sulfuric acids formed from the sulfate of ammonia, combining with the bases of the soil. In the case of Tank 3 it is accounted for, no doubt, by the calcium sulfate of the superphosphate exchanging part of its lime for the other bases, magnesia and soda, while in the case of Tank 4 it is base exchange of potash for lime, magnesia and soda.

Table 2 gives the figures for all four manurings so far as nitrogen only is concerned. Complete analyses were made of the drainage water in Periods 2, 3 and 4 also, and the results have been published elsewhere.<sup>1</sup>

In this paper we are dealing with the nitrogen change only.

The table shows that as the amount of nitrogen applied as sulfate of ammonia to Tank 2 was increased it did not lead to any increased amount of ammonia in the drainage water. As a total for Periods 1, 2 and 3, as

<sup>1</sup> Scottish Jour. of Agric., Vol. VII., pp. 8-18.

much nitrogen was recovered as ammonia in the drainage water of the unmanured tank as in the water from the tank which had received three manurings with sulfate of ammonia amounting altogether to the rate of 25 cwts. of sulfate of ammonia, or 593 lb. of nitrogen per acre. It is only when we come to Period 4 in which sulfate of ammonia was given at the rate of 20 cwt. at a single dressing, or 474.5 lb. of nitrogen, that we get a small increase in the amount of nitrogen recovered as ammonia in the drainage. In this case the amount of nitrogen recovered as ammonia was 1.25 lb. in Tank 2, while in Tank 1 it was only 0.25 lb. However, 1.25 lb. is only a very small part of the 474.5 lb. applied as ammonia. On the other hand the nitrogen in all four periods was entirely recovered as nitrate. Period 2 was a short one and lasted only from March 17th to May 22nd and as will be seen from Table 2, the nitrogen recovered as nitrate is not equal to the sum of that added as ammonia and that recovered as nitrate from Tank 1. This is made up for, however, during Period 3 in which drainage was collected over a considerably longer period than in Period 2. If we take the total of the three periods 1, 2 and 3 it will be seen that the amount of nitrogen recovered from Tank 2 as nitrate is almost exactly equal to that added to the tank as sulfate of ammonia together with that recovered from the unmanured Tank 1. Similarly in the fourth period in which a very heavy dressing of sulfate of ammonia was given to Tank 2 much more than the sum of the nitrogen added as ammonia together with that recovered from Tank 1 as nitrate is recovered in the drainage of Tank 2 as nitrate. This is accounted for by the fact that the fourth period was a very long one with an interval in the middle during which the experiment had to be discontinued for a time. The manure was applied on May 12th, drainage was collected until July 28th, the experiment was then discontinued and the tank stood covered until the following 10th of May, when drainage was again collected till August 18th. During this period the sulfate of ammonia applied to Tank 2 was itself completely subjected to nitrification and also led to increased natural production of nitrate from the humus nitrogen.

In Periods 2, 3 and 4, Tanks 3 and 4 also received sulfate of ammonia at the same rate as Tank 2. In addition, Tank 3 received superphosphate and Tank 4 superphosphate and muriate of potash. The addition of these manures led to less complete fixation of the ammonia than took place in Tank 2. A slightly increased amount of ammonia was found in the drainage of Tank 3 as compared with that obtained from Tanks 1 and 2. In the case of Tank 4 the addition of muriate of potash rendered the fixation still less complete. While the ammonia in this tank was almost as completely fixed in Periods 1 and 2 as in the case of the unmanured tank, in Periods 3 and 4 a distinct proportion, about 3 per cent, of the nitrogen added as ammonia was recovered in the drainage. In all cases however, nitrification continued very active and far the larger proportion



of the nitrogen was recovered in the drainage as nitrate. Not only so, but the total amount of nitrogen recovered as nitrate from Tanks 3 and 4 for the whole four periods was greater than the amount of nitrogen added as ammonia plus the amount of nitrogen recovered as nitrate from Tank 1. There was a difference in the rate of recovery, however, from Tanks 3 and 4. In Tank 3 the nitrate appeared more slowly in the drainage than in Tank 4 and the total amount recovered in Periods 2 and 3 was distinctly less than that found in the drainage of Tank 4. It was only in the lengthy fourth period when the collection of drainage was interrupted for about a year and was then continued again that Tank 3 began to catch up on Tanks 2 and 4. At the end of the fourth period all the three manured tanks were still producing nitrate at a more rapid rate than the unmanured Tank 1. The quantities of nitrogen found in the last two litres of drainage obtained from each tank at the end of Period 4 were compared and it was found that most nitrate was being produced by Tank 3 followed by Tanks 2, 4 and 1 in gradually decreasing amounts. Over the whole four periods it will be found that Tank 2 gave an excess of 105 lb. of nitrogen in the drainage as compared with the amount added as sulfate of ammonia plus the amount of nitrate naturally recovered from the unmanured tank. The corresponding amounts were 85.25 lb. in the case of Tank 3 and 167.5 lb in the case of Tank 4. These are very remarkable increases in the amounts of nitrate produced from the humus of the soil as compared with that obtained from the unmanured tank, and leave no doubt that the manuring of the tanks increased at the rate at which the humus of the soil was broken up to produce ammonia and ultimately nitrate. So far as the figures go they indicate that the all round manuring with nitrogen, phosphate and potash on Tank 4 had the greatest effect in promoting this extra bacterial activity in the soil with production of nitrate, while it took place most slowly in Tank 3, and, in Tank 2, which received only sulfate of ammonia, was intermediate.

The manuring of the tanks increased their acidity and at the end of the experiments the soil in Tank 4 had a pH value of 4.9. That of Tanks 2 and 3 was about 5. This is a considerable degree of acidity, yet, as the tables show, nitrification took place very actively in these soils both in the case of the added ammonia, of sulfate of ammonia, and in the case of the nitrogen of the humus matter of the soil. A considerable degree of acidity therefore is not inconsistent with active nitrification.

In other drainage experiments which have been carried out in my department, lime has been added to Craibstone soil and it has been found that the addition of lime to an otherwise unmanured soil produces an increase in the rate of nitrification in the soil as measured by the quantity of nitrate found in the drainage. In one series of experiments lime was applied to the soil in heavy dressings in gradually increasing amounts and it was found that the production of nitrate increased as the amount of lime

applied increased, and that with a heavy dressing of lime, equal to 10 tons CaO per acre, the amount of nitrate in the drainage was more than doubled as compared with an unlimed soil. The addition of lime, therefore, causes the bacterial activity in the soil to increase greatly. While this is the case, however, the experiments dealt with in this paper show that nitrification can proceed actively in a soil of such acidity as to have a pH value of 5 or a little below 5.

The conditions in these experiments are admittedly largely artificial. The tanks were filled with soil and subsoil which were, therefore, not in their natural condition but had been exposed thoroughly to the air during the filling and were not in the state of consolidation of ordinary soil and subsoil. The amount of fertilizer applied was excessive and would never be applied to a field under ordinary conditions and the fertilizers used were entirely mineral and no organic matter was applied to the soil. The water applied to the tanks was applied in an artificial way, as an amount of water equal to half an inch of rainfall was given at each application and in many cases such applications were given daily for several days one after the other.

As no organic matter was applied to the soil and no plants were allowed to grow upon it the tanks contained no organic matter in the early stages of oxidation. It is therefore very unlikely that any fixation of atmospheric nitrogen could account for the excess of nitrogen found in the drainage from the manured tanks, for these tanks, as compared with the unmanured tank, contained no organic matter which could be used as a source of energy to enable nitrogen-fixation to take place. During the first period the recovery of nitrogen in the drainage was almost quantitative.

## SUMMARY

Keeping all the above facts in mind it is difficult to resist the following conclusions: (1) nitrification can take place freely and continuously for long periods in an acid soil free from carbonate of lime, even though the acidity is gradually increasing through the excessive use of sulfate of ammonia. (2) The nitrogen of sulfate of ammonia, whether applied alone or with superphosphate and muriate of potash, even when excessively heavy dressings are given is completely, or almost completely changed into nitrate. (3) The use of mineral manures, sulfate of ammonia, superphosphate, and muriate of potash stimulated the natural bacterial changes in the soil and led to the breaking up of organic matter with formation of ammonia and ultimately the formation of nitrate, so that nitrate appeared in the drainage in excess of any nitrogen added in the manure.

These experiments give no evidence in support of the view that the nitrogen of soluble nitrogenous manures is taken up by bacteria and

stored in the soil in insoluble form in the organic matter of their bodies living and dead, especially when such manures are applied in large quantities; neither does the evidence, so far as it goes, support the view that when nitrogenous manures are applied in large quantity part of the nitrogen is lost from the soil in some gaseous form. .

#### LITERATURE CITED

- (1) Hendrick, J. *Drainage Investigations at Aberdeen* 7: 1.
- (2) ———, and Ogg, W. G. *Studies of a Scottish drift soil.* Jour. Agr. Sci. [England] 7: 458. .

# ÜBER DIE BILDUNG VON NITRITEN DURCH BAKTERIEN

E. RUNOW UND E. MISCHUSTINE

*Landwirtschaftsversuchsstation, Mauskau, U. S. S. R.*

Die Tatsache der Bildung von Nitriten aus organischen Stoffen ist von einer Reihe von Forschern (Viehover, Sack, Klein und Limberger) angemerkt worden.

Analoge Hinweise finden wir ebenfalls in den Lehrbüchern wie Z. B. von Omelianski, Kraus und Uhlenhuth u. a.

Bei dem Studium des Nitrifikationsprozesses stiessen wir auf analoge Erscheinungen. Es gelang uns aus dem Boden einige auf organischen Nährböden Nitrite bildende Bakterienarten zu isolieren. Bei der Erforschung der Fähigkeit der Bakterien zur Nitritbildung wurden verschiedene synthetische Nährböden mit verschiedenartigen Quellen sowohl Kohlenstoff als auch Stickstoffernährung angewendet.

Die Bestimmung der Nitrite wurde nach der Methode von Peter und Griess ausgeführt. Von dem ersten Autor wurden zwei Bakterien-Arten  $\alpha$  und  $\beta$  isoliert.

Die Art  $\alpha$  bildet die Nitrite auf Aminosäuren, Pepton, Brühe von Hühnereiweiss und anderen Eiweiss-Nährböden. Das Hinzufügen von Quellen Kohlenstoff-Ernährung erhöht die Intensität der Nitritbildung. Die Nitritmenge auf diesen Nährböden erreicht nach einigen Tagen 2–6 mg.  $\text{NO}_2$  auf 1 Liter; die maximale Nitritmenge erreicht in einigen Fällen bis 15 mg.  $\text{NO}_2$  nach 2 Wochen.

Die Art  $\beta$  bildet die Nitrite auf Eiweiss-Nährboden schwächer als die Art  $\alpha$ . Auf den Nährböden mit schwefelsaurem Ammonium mit verschiedenen Quellen Kohlenstoff Ernährung bilden sich nach einigen Tagen 1–5 mg. und in einigen Fällen 7–8 mg. Nitrite auf 1 Liter.

Die morphologischen Besonderheiten der oben angeführten Bakterienarten bestehen in folgendem:

Die Art  $\alpha$  stellt ein bewegliches Stäbchen von  $1.5 \mu$  mittlerer Länge dar; bildet keine Sporen; verflüssigt die Gelatine nicht; gibt auf dem Agar durchsichtige Kolonien mit charakteristischen Rändern; reduziert die Nitrate zu Nitriten.

Die Art  $\beta$ , vom Typus *Oidium*, gibt auf dem Agar hellrosa Kolonien. Die am Anfang des Wachstums sich bildenden Stäbchen von verschiedener Länge zerfallen in kurze Glieder. Die Gelatine wird von ihnen nicht

verflüssigt; die Nitrate werden zu Nitriten reduziert. Rings um den Strich auf dem Agar bildet sich allmählich eine Aureole von Auswüchsen.

Auf dem Nährboden von Winogradski, ohne Beifügung organischen Stoffe, zeigen beide Arten kein Wachstum. Die Art  $\beta$  entwickelt sich auf den Ammoniumsalzen, salpetriger Säure und Salpetersäure als Quellen Stickstoff Ernährung.

Vom zweiten Autor wurden bei dem Versuch, eine thermophile Bakterienrasse aus dem moskauischen Boden zu isolieren, die sie begleitende Mikroflora einer Untersuchung auf Fleischpepton-Agar unterworfen. Eine Reihe von Stämmen wurde auf ihre Fähigkeit, Nitrite auf organischen Nährböden zu bilden, erprobt. In zwei Fällen wurden positive Resultate erhalten. Das waren zwei nach ihren morphologischen und physiologischen Eigenschaften einander äusserst nahestehende Bazillenarten. Im Folgenden werden wir sie mit den Buchstaben A und C bezeichnen.

Zwecks Beobachtung der Nitritbildung wurden verschiedenartige synthetische Nährböden genommen. Die gewonnenen Resultate lassen sich im Allgemeinen folgendermassen zusammenfassen. Auf dem Nährboden von Winogradski (ohne organische Stoffe) wurde im Verlaufe einer längeren Zeit gar keine Entwicklung und gleichfalls keine Nitritbildung beobachtet. Auf einem seiner Zusammensetzung nach dem Nitrifikationsnährboden von Winogradski nahestehenden Nährboden, jedoch mit Hinzufügung von Glukose und Mannit, erreichen beide Arten eine üppige Entwicklung, Nitrite werden jedoch von ihnen nicht gebildet. Wenn man als Kohlenstoffquelle die Salze der Essigsäure nimmt, so findet eine Ansammlung von Nitriten in der Menge von 0.5–2.0 mg. auf 1 Liter im Laufe von 3 Tagen statt. Meistenteils überschreiten die gebildeten Nitrite quantitativ nicht 1.0 mg. in der angegebenen Zeit, zuweilen jedoch geht, wie wir hier angeben, ein energischerer Prozess vor sich. Einen ursächlichen Zusammenhang in dieser Beziehung festzustellen, ist uns nicht gelungen. Auf Nährböden, in denen Leuzin und Asparagin die einzigen Quellen der Kohlenstoff- und Stickstoff Ernährung bildeten, wurde ebenfalls die Bildung von Nitriten beobachtet. In quantitativer Beziehung erhielten wir hier eine völlige Wiederholung der Resultate der Versuche mit Essigsäure.

Eine etwas grössere Nitritmenge bildete sich bei uns auf den Nährböden mit Pepton und Eiweissbrühe. Auf der Eiweissbrühe, die ungefähr einen Monat gestanden hatte wurde die maximale 10 mg. auf 1 Liter betragende Nitritmenge erhalten.

Zum Schluss wollen wir einige charakteristische Eigenschaften der isolierten Bakterien aufführen. Dieselben sind sporenbildende Stäbchen von 1.5–3.0  $\mu$  Länge und 0.5–0.6  $\mu$  Breite. Die Lage der Stäbchen ist eine polare, die Zelle ist an der Stelle, wo die Spore sich bildet, aufgedunsen (angeschwollen).

Beide Arten geben auf dem Agar einen grauweißen feuchten Strich. Das Wachstum bedeckt die ganze Oberfläche des Agars, von der Kondensationsfeuchtigkeit beginnend. Bei einige Zeit andauerndem Stehen erscheinen auf der Kultur A kleine Knötchen in Form von Pünktchen; bei der Kultur C fehlen sie. Die optimale Entwicklungstemperatur beider Arten liegt bei ungefähr 40° C. Die Maximale Entwicklungstemperatur beträgt ungefähr 60° C.

Was den Biochemismus der obenbeschriebenen Bakterienarten anlangt, so besitzt die Nitritbildung fraglos keinen energetischen Charakter, ähnlich dem, wie es bei den nitrifizierenden Bakterien von Winogradski der Fall ist. Das lässt sich wenn auch nur daraus ersehen, dass die Menge der sich bildenden Nitrite im allgemeinen unbedeutend ist. Hier sind zwei Annahmen zulässig; entweder haben wir es hier mit einer durch die Bakterienzelle hervorgerufene Katalyse zu tun, oder die Nitrite spielen die Rolle eines Zwischenproduktes der stickstoffhaltigen Nahrung. Gegenwärtig werden von uns in dieser Richtung Untersuchungen angestellt.

Eine physiologische Eigentümlichkeit dieser Bakterienart, die unsere vorliegende Untersuchung stark erschwerte, verdient angemerkt zu werden. Das ist ihre Fähigkeit, die Nitrate zu salpetriger Säure zu reduzieren. Im Verlaufe aller von uns gemachter Versuche waren wir genötigt, die strengste Kontrolle in Bezug auf das Vorhandensein der Salpetersäure auszuüben, um der Resultate der Versuche sicher zu sein. Auf das Vorhandensein von Nitraten wurden alle Bestandteile der Nährboden geprüft und durch die zu den Versuchen dienenden Gefässe wurde ein Strom gereinigter Luft durchgeleitet. Erst diese Vorsichts-massregeln geben uns die Möglichkeit mit einer bestimmten Sicherheit von den gewonnenen Resultaten zu reden.

# NITRIFICATION IN MASSACHUSETTS SOILS

A. B. BEAUMONT

*Massachusetts Agricultural College, U. S. A.*

The first extensive study of nitrification in Massachusetts soils was begun in 1925. The study was continued through 1926 and further work is being done this year (1927)<sup>1</sup>. The investigation has considered the factors of soil type, method of soil management and cropping system. Four types of soil, representative of rather extensive areas of farm land in the State of Massachusetts, and several diverse cropping systems and methods of soil management have been studied. The data will show that under certain conditions of the investigation nitrification was intensive and nitrate accumulation very high.

## DESCRIPTION OF SOILS

The Gloucester, Merrimac and Hartford sandy loams and the Suffield silt loam were studied. The Gloucester series consists of glacial till derived mainly from crystalline rocks of granitic origin, and is probably the most extensive series in Massachusetts. Its topography varies from rolling to very rough and where not too hilly is used for dairy and livestock farming and fruit growing. In some cases the abundance of stones interferes with intensive tillage operations, but the presence of stones in the soil promotes underdrainage, which is ordinarily very good. Occasionally the presence of a semihardpan in the subsoil interferes with drainage. On account of both good air drainage and underdrainage the Gloucester series is well adapted to apple orchards and the area considered in this paper was so used. The area in question is located on the eastern slope of a drumloid having a grade of approximately 15 per cent. Mixed with the soil is a larger percentage of sedimentary rock fragments than is commonly found in Gloucester soils. Some physical and chemical characteristics of the Gloucester and other soils studied are presented in Tables 1 and 2.

The Merrimac series comprises water-worked glacial materials and occurs as old river terraces and shore lines of extinct glacial lakes or estuaries. Its surface soil is usually rather free from stones and boulders, but gravel is characteristic of the B or C horizons and occasionally appears in the surface soil; large erratic boulders very rarely are found on or just

<sup>1</sup> Credit is given Messrs. A. C. Sessions, O. W. Kelly and others for assistance in analytical work.

TABLE 1.—*Mechanical analyses of soils<sup>a</sup>**Percentage of separates*

	Diameter in millimeters						
	2.0– 1.0	1.0– 0.5	0.05– 0.25	0.25– 0.10	0.10– 0.05	0.05– 0.005	0.005–
	per cent	per cent	per cent	per cent	per cent	per cent	per cent
Gloucester sandy loam	3.6	6.9	10.0	16.3	33.8	19.7	9.7
Merrimac Do	1.0	1.7	2.7	10.2	49.4	27.4	7.6
Hartford <sup>b</sup> Do	0.0	0.4	0.7	13.6	66.4	15.1	3.8
Suffield silt loam	1.1	1.7	2.0	8.9	27.8	48.6	9.9

<sup>a</sup>Designated according to suggestion of Beaumont and Sessions, Jour. Amer. Soc. Agron. 1926. 18: 238.

<sup>b</sup>Light phase of type.

beneath the surface. The low water retentive capacity of soils of this series is shown by the promptness with which crops grown on them reflect drouthiness. With a level to rolling topography and good underdrainage the soils of the Merrimac series are highly valued for intensive farming of various kinds. Tobacco was grown on the soil here considered.

TABLE 2.—*Organic matter and hydrogen ion concentration of soils*

	Organic matter <sup>a</sup>	Reaction
	per cent	pH
Gloucester sandy loam	5.6	6.2
Merrimac Do	4.2	5.7
Hartford Do	3.9	6.1
Suffield silt loam	6.7	6.2

<sup>a</sup>Loss on ignition.

The soils of the Hartford series are composed of sediments deposited in the shallow waters of glacial lakes or estuaries, and differ from the Merrimac soils mainly in the absence of gravelly layer in the lower horizons. Their drainage is from poor to good, but never excessive as is sometimes the case with the Merrimac soils; crops rarely suffer from prolonged drouth on this series. With a level to undulating topography and freedom from stones the soils of this series are adapted to general, and some types of intensive, farming.

The Suffield series is similar in origin to the Hartford and might well be grouped with it. It consists of the finer sediments deposited in the deeper waters of glacial lakes or estuaries, only the heavier types being recognized. The rock material of this series, as well as that of the Hart-



ford and Merrimac series, is very similar to that of the Gloucester from which it has been derived through weathering and erosion. The resistant mica particles originally present in the parent rock material are much in evidence in the Suffield series. Drainage of this series is usually poor, thus limiting it, unless artificially drained, very largely to grass crops. Maize was grown on the area studied.

### FERTILIZER AND CULTURAL PRACTICES

On the Gloucester soil is located Block G-H. This block, consisting of four plots of approximately  $\frac{1}{4}$  acre each, was planted to apple and pear trees in 1911. The present soil management system was begun in 1922. The trees of two plots have been continuously mulched while the soil of the other two plots has been cultivated until midsummer and then sown to a cover crop annually. No fertilizer has been applied. To the mulched plots a mulch composed of waste hay and straw has been applied in the spring and fall of each year at the rate of 6 to 8 tons per acre; the fall application was omitted in 1926. The mulch was placed mainly under the overhanging branches and by 1926 had become so abundant that weeds and grasses were almost wholly absent. The lower part of the mulch was well humified in 1926, thus serving as a constant supply of nitrifiable material. Since the beginning of the experiment the mulch has not been removed nor the soil plowed; in this respect the experiment differs from those heretofore reported (1, 2).

On the Merrimac soils are located a series of plots devoted to a study of methods of soil management for tobacco growing. Only two treatments will be considered: tobacco grown continuously since 1923 with fertilizer but (1) with and (2) without animal manure. The plots are  $\frac{1}{25}$  acre in area. A mixed fertilizer containing 5 per cent  $\text{NH}_3$ , 4 per cent  $\text{P}_2\text{O}_5$  and 5 per cent  $\text{K}_2\text{O}$  has been applied annually at the rate of 3000 lb. per acre. Cottonseed meal has furnished a large proportion of the nitrogen. The soil is spring plowed and finally fitted with fertilizer applied broadcast the latter part of May. Tobacco plants are usually set about the first of June. Intense cultivation of tobacco fields is the rule.

On the Hartford series tobacco was grown on one field and maize on another, in 1925. The fertilizer and cultural practices for tobacco on this soil were very similar to those described for the Merrimac soils except as follows: (a) the fertilizer mixture was differentiated into two grades, (A) one high in organic nitrogenous material and (B) another high in inorganic sources of nitrogen (Table 7); (b) two plots were grown with a cover crop of timothy and two without a cover crop.

For maize grown on Hartford and Suffield soils fertilizer and cultural practices common in Massachusetts were followed. The manured soils received some 15 tons per acre of animal manure applied in the winter and early spring and 250 lb. 16 per cent superphosphate applied at planting

time. The fertilized (Table 9) soil received 400 lb. of mixed fertilizer containing 3 per cent  $\text{NH}_3$ , 10 per cent  $\text{P}_2\text{O}_5$ , 6 per cent  $\text{K}_2\text{O}$  applied at planting time, but no manure.

### CLIMATIC CONDITIONS

The climatic conditions prevailing during the period of the investigation were rather typical of those of the northeastern United States; the mean annual precipitation at Amherst being 44.17 inches rather evenly distributed through the year, and the mean temperature 46.8° F. A comparison between precipitation during the period of the investigation and

TABLE 3.—Seasonal temperature and precipitation at Amherst, Massachusetts<sup>a</sup>

Month	Mean air temperature <sup>b</sup>	Mean precipitation <sup>a</sup>	1925 Precipitation	1926 Precipitation
	°F.	in.	in.	in.
Jan.	23.8	3.38	3.42	3.23
Feb.	22.3	3.27	3.64	5.01
Mar.	34.5	3.60	4.12	3.95
Apr.	45.7	3.18	3.10	3.62
May	57.0	3.80	2.55	1.19
June	64.6	3.61	4.28	2.03
July	71.0	4.40	6.97	3.24
Aug.	68.4	4.36	1.93	3.97
Sept.	61.4	3.66	3.09	1.50
Oct.	51.5	3.71	4.74	5.02
Nov.	38.3	3.62	3.23	5.38
Dec.	22.8	3.58	3.56	2.78
Annual	46.8	44.17	44.63	40.92

<sup>a</sup> Data from Summary of Climatological Data for the United States by Weather Bureau, U. S. D. A., and Monthly Bulletins of Mass. Agr. Expt. Sta.

<sup>b</sup> For 20 yr.

<sup>c</sup> Do 84 Do

mean precipitation can be had from Table 3. It may be noted that the total precipitation of June and July 1925 was considerably above, while that of 1926 was considerably below the mean for the same period. This difference in precipitation in the two seasons probably accounts in a measure for the difference in nitrate accumulation to be shown later.

### METHODS

Nitrate determinations were made on composite soil samples drawn at weekly intervals during the active growing seasons or at other intervals as indicated later. In sampling, twenty or more borings were drawn from a depth of 7 inches with a soil auger (diam. 1.5 inches) and mixed together

for a composite sample; on the mulched plots the mulch was pushed aside when borings were made. The soil samples were immediately taken to the laboratory and nitrates determined by the phenol di-sulfonic acid method. Moisture was also determined at the time nitrate determinations were made, and the temperature of field soils was taken at the time of sampling, but these data are not recorded here.

## LITERATURE

The extensive literature bearing on nitrification in soils will not be reviewed here. For summaries of the literature and bibliographies the reader is referred to the writings of Albrecht (1), Scott (2), and Smith (3).

## RESULTS AND DISCUSSION

Soil nitrates expressed in p.p.m.  $\text{NO}_3$  on the basis of the oven-dry weight of soil are reported in Tables 4 to 9.

It is evident from the data that the lighter types of Massachusetts soils studied have high nitrifying power under certain favorable conditions. Just what maximum accumulation of nitrates might be attained in the

TABLE 4.—*Soil nitrates, Gloucester soil, 1925*

*Parts per million  $\text{NO}_3$ , dry weight basis*

Cultivated				Mulched		
Date	G	H	Average	G	H	Average
5/8	Trace	11.6	5.8	29.1	13.1	21.1
15	5.9	11.8	8.9	18.3	6.2	12.3
22	3.0	7.2	5.1	10.4	14.0	12.2
29	4.3	17.7	11.0	15.8	9.6	12.7
6/5	3.7	18.7	11.2	28.4	14.4	21.4
12	4.2	11.6	7.9	24.6	60.5	42.6
19	7.3	35.3	21.3	36.5	41.9	39.2
26	14.9	36.2	25.6	32.9	34.6	33.8
7/3	9.4	26.6	18.0	21.2	18.0	19.6
10	5.1	21.4	13.3	19.7	23.5	21.6
17	7.3	28.4	17.9	48.8	40.0	44.4
24	4.4	9.4	6.9	25.4	32.5	29.0
31	4.4	6.0	5.2	23.6	18.4	21.0
8/7	11.1	10.9	11.0	49.9	95.5	72.7
14	3.1	21.0	12.1	67.0	34.8	50.9
21	8.1	16.7	12.4	40.9	56.0	48.5
28	1.3	5.7	3.5	34.9	66.2	50.6
9/4	1.4	5.5	3.5	96.6	81.1	88.9
25	1.5	1.4	1.5	63.4	53.2	58.3
10/16	Trace	1.3	0.7	38.1	77.5	57.8
Average	5.6	15.2	10.1	36.3	39.6	37.9

heavier Suffield silt loam under more favorable conditions cannot be determined from the data at hand.

TABLE 5.—*Soil nitrates, Gloucester soil, 1926*

*Parts per million NO<sub>3</sub>, dry weight basis*

Cultivated				Mulched		
Date	G	H	Average	G	H	Average
5/10	3.3	8.5	5.9	32.7	23.8	28.3
17	4.4	6.4	5.4	35.6	35.6	35.6
24	4.3	8.1	6.2	69.9	67.0	68.5
6/1	3.3	3.6	3.4	91.0	54.6	72.8
8	3.5	3.7	3.6	97.5	93.5	95.5
15	3.3	41.5	22.4	73.2	44.3	58.8
22	5.4	13.1	9.3	88.3	92.5	90.4
29	17.0	30.5	23.8	133.0	160.0	146.5
7/6	79.2	87.0	83.1	378.0	527.0	452.5
13	38.8	103.0	70.9	265.0	387.0	326.0
20	14.5	47.2	30.9	205.0	87.0	146.0
27	19.0	34.3	26.7	205.0	87.5	146.3
8/3	14.7	46.5	30.6	171.0	51.0	111.0
10	12.7	34.5	23.6	267.0	62.2	164.6
17	3.1	3.2	3.2	36.6	58.1	47.4
24	Trace	13.6	6.8	177.0	64.9	121.0
31	10.4	15.7	13.1	160.0	108.0	134.0
9/7	Trace	18.5	9.3	111.0	132.0	121.5
14	Do	Trace	Trace	65.8	111.0	88.4
21	Do	Do	Do	171.0	169.0	170.0
28	Do	Do	Do	210.9	159.0	185.0
10/5	Do	23.1	11.6	157.0	101.0	129.0
19	Do	2.9	1.5	320.0	249.0	284.5
11/2	Do	Trace	Trace	156.8	162.3	159.6
17	Do	Do	Do		169.0	84.5
12/2				104.0	74.6	89.3
14				109.3	30.4	69.9
30				65.9	56.0	61.0
1/15				7.0	11.5	9.7
Average	14.8	27.3	19.6	141.2	118.2	127.5

A direct comparison between soil types and nitrification is possible only with the manured plots of Hartford sandy loam and Suffield silt loam. (Table 9). Here with equivalent fertilization and the same crop there was very little difference on the whole, although a maximum of 101 p.p.m. was reached in the lighter soil as compared with 84 in the heavier soil and the maximum came earlier in the season in the former soil. Because of differences in cropping systems, fertilization, or seasons no direct comparison can be made between other soil types as to their nitrifying power.

TABLE 6.—*Soil nitrates, Merrimac soil, tobacco, 1926**Parts per million NO<sub>3</sub>, dry weight basis*

Date	Without manure <sup>a</sup>	With manure <sup>a</sup>
6/9	161	341
6/17	341	522
6/24	289	314
7/1	405	397
7/8	673	797
7/15	364	375
7/22	325	373
7/29	292	334
8/5	179	225
Average	336.6	408.7

<sup>a</sup>5-4-5 fertilizer applied to each plot, 3000 lb. per acre

The kind and amount of material added to the soil as a fertilizer amendment or mulch, and the method of soil management, are shown by data in Tables 4, 5, 7, 8 and 9 to be very important factors in nitrification and

TABLE 7.—*Soil nitrates, Hartford soil, tobacco, 1925 (no cover crop)**Parts per million NO<sub>3</sub>, dry weight basis*

High organic				Low organic		
Date	62A	68A	Average	62B.	68B	Average
5/4	94	44	69	66	27	47
5/11	85	49	67	89	37	63
5/18	93	55	74	117	37	77
5/25	104	54	79	104	56	80
6/1	118	100	109	145	61	103
6/8	80	67	74	117	68	93
6/15	97	67	82	108	61	85
6/22	187	106	147	192	114	153
6/29	236	179	208	304	186	245
7/6	359	311	335	436	303	370
7/13	327	275	301	326	312	319
7/20	256	224	240	338	284	311
7/27	144	196	170	247	75	161
8/3	151	74	113	234	44	139
8/10	158	48	103	231	24	128
8/17	104	11	58	68	7	38
8/24	128	29	79	135	35	85
8/31	108	38	73	114	23	69
9/7	36	21	29	49	20	35
Average			127.9			136.9

nitrate accumulation. In fact, they are paramount in these soils. These factors, moreover, are more or less interwoven with the cropping system. Compare, for example, the high results from the mulched as compared with the cultivated soil as shown in Tables 4 and 5. Also compare the high accumulation of nitrates in Hartford sandy loam growing tobacco with the small amount in the same type of soil growing corn, both crops receiving commercial fertilizer but the former receiving 150 lb. of fertilizer ammonia as compared with only 12 for the latter. (See Tables 7, 8, and 9). Here, however, there is also a crop difference.

TABLE 8.—*Soil nitrates, Hartford soil, Tobacco, 1925 (Timothy cover crop)**Parts per million NO<sub>3</sub>, dry weight basis*

High organic				Low organic		
Date	59A	65A	Average	59B	65B	Average
5/4	97	82	90	45	31	38
5/11	87	78	83	60	65	63
5/18	106	126	116	91	87	89
5/25	109	89	99	112	93	103
6/1	141	139	140	114	108	111
6/8	134	121	128	90	129	110
6/15	179	158	169	86	126	106
6/22	233	270	252	179	200	190
6/29	279	308	294	274	238	256
7/6	456	434	445	408	394	401
7/13	422	509	466	412	432	422
7/20	340	314	327	320	305	313
7/27	159	102	131	162	85	124
8/3	162	119	141	143	90	117
8/10	132	168	150	133	73	103
8/17	86	45	66	136	68	102
8/24	90	136	113	117	49	83
8/31	85	136	111	56	40	48
9/7	33	51	42	31	25	28
Average			177.0			147.7

Under the condition of an abundant supply of fertilizer nitrogen the Merrimac soil growing tobacco in 1926 is seen to produce and accumulate a large amount of nitrates; 673 p.p.m. with fertilizer only and 797 with fertilizer plus manure (Table 6).

The high maximal accumulation as shown with the 1926 tobacco on Merrimac soil is also related to the climatic conditions of that year. June and July were comparatively dry months—moisture enough to promote nitrification but dry enough to allow accumulation. Our moisture data showed that nitrification would occur at the low figure of 9 per cent

(dry-basis) moisture in light sandy soils. Also, high nitrification occurred in light soils containing as much as 47 per cent water. The maximal accumulation (797 p.p.m.) in tobacco soil occurred with a moisture content of 20.2 per cent.

Some evidence of the relation of applied organic matter to nitrification and "nitraccumulation" (proposed for nitrate accumulation) is found in Tables 4 and 5, mulched versus cultivated; Tables 7 and 8, high organic versus low organic fertilizer and cover crop versus no cover crop; Table 9, manured versus fertilizer.

TABLE 9.—*Soil nitrates, Suffield soil, maize, 1925*

*Part per million NO<sub>3</sub>, dry weight basis*

Hartford sandy loam			Suffield silt loam
Date	Manured	Fertilized	Manured
5/15	63	6	29
22	78	12	54
29	55	15	73
6/5	44	21	55
10	61	19	47
19	101	40	52
26	50	31	75
7/3	18	12	84
10	12	6	15
17	7	9	24
24	2	2	7
31	2	tr	6
8/7	5	1	3
14	2	tr	2
21	3	2	2
28	4	1	3
9/4	4	tr	2
18	9	1	3
10/3	21	4	2
Average	28.5	9.6	28.3

With the mulched versus cultivated we have a unique and extreme case of applied organic matter.<sup>1</sup> From the data it is very evident that the large amount of organic matter thus applied is very effective in causing "nitraccumulation." In the case of the tobacco fertilizer with high and low amounts of organic carriers there is no significant difference. The effect of the timothy cover crop plowed under was slightly to depress nitrification. The effect of manure (15 to 20 tons per acre for corn, 8 tons for tobacco) was to increase nitrification.

<sup>1</sup> A detailed report of this experiment will soon appear in Soil Sci.

Fertilizer nitrogen appears to have been much more effective, pound for pound, in promoting nitrification than was the nitrogen of manure or other forms of organic matter. This relation is shown by the data in Tables 7 and 9 which show nitracumulation under very similar nitrogen applications. How much, if any, of this higher accumulation with the fertilizer is due to a stimulating effect of the fertilizer, is not known. That such stimulation may be expected is shown by the report of Smith (3).

Very similar to the data of several other workers ours show a maximum of nitrate accumulation the latter part of June or the first part of July. The peak of the curve for tobacco soils is especially pronounced. This is probably closely related to the nature of the growth of the plant. Tobacco is usually set about June 1 and attains sufficient size to draw heavily on nutrients of the soil about July 1.

### SUMMARY AND CONCLUSIONS

Nitrification and "nitracumulation" (proposed for nitrate accumulation) have been studied in four rather different types of Massachusetts soils.

The Gloucester, Merrimac, and Hartford sandy loams and the Sufield silt loam were studied. These are described in detail. A wide range of edaphic, topographic, fertilization, cultural and cropping conditions are covered in the investigation.

Data on soil nitrates are presented which show:

The light, well drained Massachusetts soils typified by three of those studied have a high nitrifying efficiency under favorable conditions.

Characters determining soil type are important as affecting nitrification, but for the range of conditions studied, the kind of soil management and manurial system employed are much more important.

### LITERATURE CITED

- (1) Albrecht, W. A. 1922. Nitrate accumulation under a straw mulch. *Soil Sci.* 14: 299.
- (2) Scott, H. 1921. The influence of wheat straw on the accumulation of nitrates in the soil. *Jour. Amer. Soc. Agron.* 13: 233.
- (3) Smith, A. W. 1927. A study of the factors influencing the efficiency of different forms of nitrogen as related to soil type and cropping system in the Atlantic Coastal Plain Region. Part I, *Soil Sci.* 23: 137.



# A L'ÉCOLOGIE DE BACTÉRIES DU SOL, QUI PRODUISENT LA FERMENTATION DE L'URÉE

E. MICHOUSTINE

## INTRODUCTION

Le savoir de l'écologie et en particulier de la géographie écologique de la microflore du sol, possède à côté de l'intérêt purement théorique, aussi une certaine importance pratique, quand il s'agit de l'appréciation du sol au point de vue bactériologique.

L'auteur de l'article présent a fait la tentative d'élaborer une méthode, qu'éclaire plus complètement la question touchée, que celle qu'existe déjà. Beaucoup de réactions microbiologiques acceptées pour les analyses ordinaires, somment le travail d'une série de groupes physiologiques de bactéries. Leur différenciation pouvait être dans beaucoup de cas utile et démonstrative. En prenant pour base les réactions microbiologiques dans telle ou telle modification (Winogradski, Waksman et autres) l'auteur recommande sa méthode, en qualité d'auxiliaire et complémentaire.

Pour le moment elle est travaillée seulement pour le groupe de bactéries faisant fermenter l'urée. Cette méthode élaborée après une série d'expériences préliminaires est fondée là-dessus. On crée une série de milieux électives différents quant aux sources nutritives carbonées et nitrées. Dans le cas donné, comme source d'azote était prise l'urée (2 pour cent), comme source de carbon les sucres différents, les esprits et les sels des acides organiques (0.5 pour cent). Comme base fondamentale servait le milieu de Winogradski sans sulfate d'ammoniaque et une dose diminuée de carbonate de chaux. On a aussi essayé de varier le nourrissement nitré. On ajouta aux milieux les sels d'ammonium, l'asparagine, l'extrait de viande.

On stérilisa les milieux à l'urée, ce que créa une certaine alcalinité élective pour le groupe de bactéries étudiées. Pendant l'essai chaque milieu fut infecté par un petit morceau du sol frais et on le met a 30° pour 3 à 4 jours. Pendant ce temps s'opérait une forte décomposition de l'urée. Après de délai de temps on ressemait de chaque tube dans 3 à 4 tubes pareils. On le faisait en se fondant sur ce que les premiers tubes ne donnaient pas la possibilité de faire de conclusions déterminées.

Là bas les substances extractives du sol en passant dans la solution, peuvent faire se développer une microflore, laquelle n'est pas capable de se développer dans leur absence.

En ressement nous nous délibérons de cette flore accompagnante et en

recevant une caractéristique pour ce milieu. Les seconds tubes furent tenus au thermostat pendant une semaine, dans 3 et 7 jours on titra 0.1 N acide sulfurique à l'acide rosalique pour le compte détaillé de l'urée décomposée.

Ce de cette manière qu'on établissa la relation entre la microflore du sol et les milieux différents. C'est vrai qu'ici nous prenons en égard le mélange de formes bactériaux, mais bien plus spécifique par leurs particularités physiologiques.

Cette méthode peut avoir l'air d'être trop volumineuse par l'abondance de milieux nécessaires pour l'analyse. Néanmoins, comme cela ce voit de résultats on peut choisir 5 à 6 de plus caractéristiques et baser sur eux l'expérience.

### LE CARACTÈRE DE SOLS EXPLORÉS

Pour le travail actuel fut prise une série de sols fortement distinct entre eux. Nous avons utilisé de préférence les sols du département de Moscou, quoique le travail fut aussi mené avec les sols du sud. D'ailleurs avec ces, derniers il n'est pas encore terminé.

Pour la commodité de l'orientation dans les résultats obtenues nous divisions les sols analysés en quelques groupes:

1. Sols de prés en fonds de rivière
  - (a) du type de prés (Nrs. 7, 8, 9, 10);
  - (b) envasement sableux, faiblement couvert d'herbes (Nr. 11)
2. Sols de prés de vallées desséchées
  - (a) à réaction moins acid (Nrs. 5 et 6);
  - (b) à réaction acid (Nr. 4)
3. Sols de bois de sapin et pin (Nrs. 1, 2 et 3)
4. Sols cultivés, ordinairement engraisser par le fumier (Nrs. 12, 13 et 14).

Le dernier groupe des sols est quelque artificiel, nous l'avons mis à part, parceque il fut soumis à la réaction monotype de l'homme.

### LES RÉSULTATS DE L'EXPLORATION

Nous commencerons notre revue par les *sols de bois de pins et sapins*, le plus pauvres en microflore.

Ces sols en général extrêmement acides causaient la décomposition de l'urée seulement sur le milieu de bouillon de viande. Les autres milieux donnent la réaction négative pendant toute la durée de l'expérience, restant tout à fait limpide après le second ressement.

Les *sols de prés de vallées desséchées* du type acide (Nr. 4) donnent une réaction presque identique à la précédente (bois de sapin).

Quand aux sols plus alcalins (Nrs. 5 et 6) ils montrent une réaction positive sur l'acide pommique et citrique, et sur l'asparagine. L'alca-

TABLE 1.— *L'alcalinité de milieux sous l'influence du développement*

No. d'ordre	Caractère du sol	Bouillon de viande		Asparagin		Acide citrique		Acide pommique	
		3 jours	7 jours	3 jours	7 jours	3 jours	7 jours	3 jours	7 jours
1	Bois de pin dans l'entourage de Moscou Pré de l'endroit No. 1	4,8		0,1	0,18	0,03	0,02	0,0	0,0
2	Sol couvert de mousse tout près du ruisseau	4,2		0,1	0,1	0,02	0,00	0,0	0,28
3	Bois de sapin Pré de l'endroit No. 1	5,6		0,1	0,15	0,02	0,04	0,2	0,25
4	De buissons rares, le lieu où sont tendus les filets et les graminées	5,1		0,48	0,68	0,02	3,0	0,06	0,30
5	La vallée desséchée, près de l'endroit No. 7	5,4		0,05	0,38	0,03	0,92	0,3	2,2
6	Un pré sur la tourbière, dans les environs de Moscou	5,3		1,48	2,0	0,23	3,4	1,3	3,1
7	Un pré en fonds de rivière; le lieu où sont tendus les filets (O-KA)	4,8		0,58	0,68	0,58	1,3	1,58	2,0
8	Un pré en fonds de rivière; le centre de pré	4,7		0,53	0,9	1,0	2,5	2,2	2,7
9	Do pré, s'approchant du fleuve	5,2		0,63	1,1	1,1	3,3	0,6	1,7
10	Le centre de pré en fonds de rivière	4,7		1,7	2,85	0,33	1,1	0,8	1,8
11	Moscou	4,6		0,33	0,6	0,48	0,6	2,5	2,3
12	Évasements sableux de la rivière	4,9		2,4	3,1	1,0	3,2	3,4	2,7
13	Le terrain de la Station Bacteriol.—Agronomique	3,2		2,7		0,7		3,2	
14	Un jachère à l'Académie agronomique de Moscou	3,4		2,4		1,1		3,0	
	En même endroit un champs sous l'avoine								

linité sur l'asparagine en cas de sol Nr. 5 peut être expliqué par la décomposition de l'asparagine même.

Il faut noter qu'en général, l'augmentation de l'alcalinité est beaucoup plus considérable sur l'acide pommique que sur l'asparagine. On peut l'expliquer par l'action dépressive de la microflore accompagnante, laquelle peut ressortir plus en relief sur l'asparagine. Le sol Nr. 6 de type de prés présente une microflore plus riche, donnant le moins sur l'acide oxalique, les sucres et sur de la glycérine.

En général nous nous formons l'opinion définitif, se basant sur le travail avec les sols du nord, que les sols vierges sur les sucres ne donnent pas de réaction positive.

Au premier moment il peut sembler bizarre que la glucose et les autres sucres sont plus mal usés que les sels d'acides organiques.

Néanmoins nous en trouvons la confirmation chez Christensen (1), Roubentschik (2) et autres.

Le microflore de prés en fonds de rivière montre que la faible capacité pour elle du milieu à la glycérine. Aussi faiblement est utilisée la glucose et l'acide oxalique. Il est bizarre qu'on y obtient une réaction positive sur le milieu du sucre de lait. Celle est aussi le développement quoique

de la microflore en cc. 0.1 N acide sulfurique pour 1 cc. de milieu

Acide acétique		Acide tartrique		Acide oxalique		Glycérine		Glucose		Sucre de lait		pH
3 jours	7 jours	3 jours	7 jours	3 jours	7 jours	3 jours	7 jours	3 jours	7 jours	3 jours	7 jours	
0,03	0,02	0,02	0,12	0,0	0,08	0,0	0,06	0,0	0,11	0,0	0,0	.
0,0	0,0	0,0	0,0	0,03	0,03	0,0	0,0	0,0	0,0	0,3	0,2	4,18
0,0	0,0	0,0	0,03	0,03	0,1	0,0	0,02	0,0	0,0	0,0	0,02	3,3
0,02	0,0	0,02	0,0	0,4	0,18	0,0	0,07	0,0	0,0	0,13	0,2	4,01
0,03	1,5	0,0	0,0	0,0	0,0	0,0	0,1	0,0	0,0	0,0	0,02	5,09
0,06	2,3	0,1	1,1	0,02	0,1	0,0	0,2	0,0	0,0	0,08	0,1	5,31
0,15	1,78	0,08	1,1	0,03	0,26	0,05	0,07	0,05	0,58	0,68	1,1	5,76
0,28	2,6	0,4	2,1	0,13	0,35	0,03	0,08	0,04	0,45	0,33	1,0	6,63
0,23	2,3	0,2	1,0	0,1	0,31	0,0	0,12	0,0	0,34	0,33	1,7	
0,0	2,6	0,0	1,3	0,03	0,4	0,03	0,38	0,03	0,78	0,0	1,5	6,61
0,06	0,58	0,0	1,1	0,23	0,33	0,0	0,03	0,0	0,13	0,0	0,02	6,68
1,0	2,8	1,7	3,3	0,28	1,0	2,1	3,2	2,65	3,7	3,5	2,4	6,76
1,0	2,6	2,0				1,05		2,85		3,75		5,3
2,1	2,8	1,8				2,0		2,35		3,85		

faible sur les milieux avec la glucose est en contradiction avec la position émise, constitutante en ce que les sols vierges ne donnent sur ces milieux des réactions positives. Nous croyons cependant que cela s'explique par le fait qu'au printemps les prés reçoivent par l'inondation avec l'envasement la microflore de champs cultivés; ces sols comme nous le verrons plus loin sont extrêmement riches d'une microflore diverse.

Quand on infecte un milieu de glucose par le sol de prés, là bas peuvent se développer énergiquement, en présence des substances extractives, des bactéries qui ne décomposent pas l'urée.

Si les bactéries qui font fermenter l'urée, capables de se développer sur de la glucose et importées des champs cultivés, sont en petit nombre on peut admettre le fait de leur dépression.

Le sucre de lait est une source bien plus pire et bien moins spécifique. Voilà pourquoi sur ce milieu les bactéries qui font fermenter l'urée, peuvent se mettre au dessus des accompagnantes.

*Les sols cultivés* évoquent le développement et la décomposition de l'urée dans tous les milieux. La fumier toute pourrie répète entièrement la réaction de ces sols, ainsi que nous y voyons son influence.

Basé sur les réactions étudiés, les singularités spécifiques de sols se

TABLE 2.—*La table schematique de résultats obtenues dans la table 1 \**

No. d'ordre	Bouillon de viande	Asparagin	Acide citrique	Acide pommique	Acide acétique	Acide tartrique	Acide oxalique	Glycérine	Glucose	Sucre de lait	pH
1	++	—	—	—	—	—	—	—	—	—	
2	++	—	—	—	—	—	—	—	—	—	4,18
3	++	—	—	—	—	—	—	—	—	—	3,3
4	++	±	+	±	—	—	—	—	—	—	4,01
5	++	±	+	+	+	—(†)	—	—	—	—	5,09
6	++	++	+	++	+	+	—	—	—	—	5,31
7	++	±	+	++	+	+	±	—	±	+	5,76
8	++	+	++	++	+	+	±	—	±	+	6,63
9	++	+	++	+	+	+	±	—	±	+	
10	++	++	+	+	+	+	±	±	±	+	6,61
11	++	±	±	++	+	±	±	—	—	—	6,68
12	++	++	++	++	++	++	+	++	++	++	6,76
13	++	++	++	++	++	++		++	++	++	5,3
14	++	++	++	++	++	++		++	++	++	

\* — Le 1 et 2 titrement donnent a peu près 0,2 cc. et quelquefois moins.

± Le 2 titrement donne a peu près 0,7 cc.

+

++ Le 2 titrement donne a peu près 1 cc.

++ Le 1 titrement donne 1,0 cc.; le 2 titrement donne plus.

démontrent caractéristiques. L'étude par voie pareil d'autres réactions microbiologiques rendra, il est possible, un service important au cours de l'appréciation des sols au point de vue microbiologique.

#### LITTERATURE CITÉE

- (1) Christensen. 1910. Centbl. Bakt. (Etc.) Abt. 2. 27: 336.
- (2) Roubentschik. 1926. Ibid. 68: 161.

# THE CHEMISTRY OF HUMUS FORMATION

O. SCHREINER AND P. R. DAWSON

*United States Department of Agriculture, U. S. A.*

## INTRODUCTION

Not long after the humus substances of the soil first attracted the attention of chemists in the latter part of the 18th century, it was noted that more or less similar substances likewise occurred, not only in other natural sources, but also as the artificial products of certain reactions involving organic compounds. The subsequent development of organic chemistry has revealed a number of such reactions yielding, under laboratory conditions, a variety of these artificial "humus," "humic acid" or "humin" substances, more or less similar in their physical and chemical properties to each other and to the naturally occurring substances. While arousing the interest of a limited number of investigators, they have, for the most part, represented unwelcome by-products of laboratory experiments or technical processes; on account of their amorphous nature, limited solubility, and generally ill-defined properties, they have not proved attractive or fruitful objects of investigation. In recent years, however, the realization of the importance of maintaining or restoring the organic matter of soils, the efforts to produce "artificial farmyard manures" from crop wastes, and the interest in the related problems of coal formation have stimulated further study of the reactions leading to artificial "humus"-like substances, with the result that more light has been shed upon the processes involved.

The variety of such reactions yielding more or less similar products suggests that the naturally occurring substances are rarely homogeneous products of any one type of reaction or any one group of parent substances, but that they represent accumulations of the more resistant end-products of a variety of reactions taking place under natural conditions, either directly or indirectly through biological processes. This conception emphasizes the difficulty of determining with any precision, by study of the end-products as we find them in soils and other natural sources, the reactions and intermediate substances involved in the formation of humus from plant or animal remains. On the other hand, the study, under controlled conditions, of the different types of reactions yielding "humus"-like products from specific decomposition products of plant and animal remains, offers the promise of establishing some of the stages in such transformations and of yielding a clue to the nature of the reactions and

intermediates in the more complex natural environment. Likewise it may enable us to anticipate their presence and their relation to the biological, chemical and physical cosmos of the soil.

With this end in view, a survey of data accumulated in the literature and experimental studies of several types of such reactions have been in progress in this laboratory, with particular emphasis upon the mechanism of the reactions and less regard for the specific nature of the poorly defined end-products themselves.

### FORMATION OF HUMUS-LIKE SUBSTANCES FROM CARBOHYDRATES

Of the groups of organic compounds readily yielding in the laboratory such dark-colored, amorphous products, one of the most familiar is that of the general class of carbohydrates. As typical of this class may be taken the action of aqueous solutions of mineral acids upon certain common hexose sugars. The products of such reactions have been studied by a number of earlier and more recent experimenters, with more or less indifferent success in contributing to our knowledge of the intermediate reactions involved. Of these studies may be cited those of Braconnot (13), Boullay (12), Malaguti (41), Bouchardet (11), Péligot (50), Stein (57), Mulder (49), Sestini (56), Udransky (60), Berthelot and André (7), Troussov (59), Bottonley (10), Robertson, Irvine and Dobson (54), and others. Other investigations concerned to a greater extent with the intermediate reactions and compounds than with the humus-like end-products have shed some light upon the mechanism of these "humus-forming" reactions, at least in the earlier stages. Here may be mentioned the contributions of Grote and Tollens (33, 34), Conrad and Guthzeit (15, 16), Hoppe-Seyler (38), Düll (18), Kiermayer (39), Fenton and Gostling (24), Beckley (6), Marcusson (42, 43, 44, 45, 46, 47), Hibbert (36), Eller (20), Punmerer and Gump (51), and others.

To take a specific example, it is a familiar fact that warming a dilute hydrochloric acid solution of sucrose results in the rapid darkening of the color and finally in the precipitation of copious amounts of brown to black flocculent "humus" or "humin" material. The evidence accumulated in the above-mentioned sources and furnished by experiments in this laboratory indicates that (after hydrolysis of the disaccharide) the furan aldehyde, hydroxymethylfurfural, is the first intermediate formed, the levulose component of the invert sugar yielding the compound much more rapidly than the glucose. This highly reactive compound then undergoes more profound decomposition, yielding, apparently in parallel reactions, equimolecular quantities of levulinic and formic acids on the one hand, and the "humus"-like material on the other. The former involves rupture of the furan ring; whether or not the latter is the result of condensation of the intact aldehyde or of condensation of a highly reactive

transition product formed on rupture of the ring, but recondensing before splitting into formic and levulinic acids, is not yet established. There are reasons for assuming that the latter is the case.

From this point on the process of condensation is so rapid that it has proved impossible to isolate any specific homogeneous intermediate product. It seems to be a question of rapid piling up of molecular weight, with more or less increase in the percentage of carbon in the aggregate. The earlier stages show a high degree of solubility or dispersibility in the acid solution, although the products may be precipitated by such reagents as basic lead acetate, or extracted with certain organic solvents. As the degree of condensation increases they are precipitated in a flocculent form from the acid solution, but still show a high degree of dispersibility in alkaline media ("humic acids"). At a later stage they become more or less completely insoluble or non-dispersible in any medium ("humins"). There does not seem to be any definite stage of reaction that may be halted at a definite degree of condensation, although adherence to identical arbitrary conditions may yield more or less identical mixtures. If the reaction is continued long enough the products will be preponderantly of the "humins" type. Even the last-named may gradually undergo further condensation and oxidation on keeping for some time. Hence any "fractions" separated on the basis of solubility, color, etc., are but arbitrary selections of mixtures; the numerous attempts that have been made to identify them chemically or to assign them a constitution on the basis of their empirical composition are of little significance.

The more complex carbohydrate substances, such as starches, gums, celluloses, etc., undergo much the same transformations after breakdown into their simpler monose derivatives by hydrolytic processes. In these cases while the process may be relatively gradual the intermediates may react so readily as to escape detection.

In the case of the pentoses or methylpentoses, or their polymers, an analogous process prevails. Here furfural, or methylfurfural respectively, is the furan intermediate in place of hydroxymethylfurfural, as in the case of the hexoses.

## HUMUS-LIKE SUBSTANCES FROM PHENOLIC COMPOUNDS

Another familiar type of artificial "humus"-like substances is derived from certain phenolic compounds or the related quinones under a variety of conditions favoring oxidation. The most common instances are found in the case of alkaline solutions of pyrogallol, hydroquinone, catechol, etc., where oxygen is more or less rapidly absorbed, with darkening of the color and ultimate formation of dark brown or black "humus"-like material ("humic acid"), largely dispersible in alkali but precipitated by acid. The presence of oxidizing agents, such as persulfates, greatly accelerates the reaction. Likewise ordinary para-benzoquinone, particularly in the



presence of traces of impurities, undergoes darkening on the surface of the crystals, with the ultimate production of amorphous black material; the same occurs in water suspension, particularly on warming.

Limiting the discussion to the simpler phenols, the oxidation of these compounds and their related quinones has been extensively studied by many investigators. Among those who have attacked the problem from the point of view of the "humus"-like substances formed may be cited Stoltzenberg (58), Hoppe-Seyler (38), Hofmann (37), Adler (3, 4), Eller and his co-workers (19, 20, 21, 22, 23) and others. The evidence thus accumulated and the general experimental data available on the subject of phenol oxidation indicate that the compounds of this class, which contain the phenolic hydroxyl groups in the ortho and para-positions with respect to each other are most readily reactive, the ortho-compounds exceeding the para-compounds in this respect. Examples are furnished by catechol and hydroquinone, respectively. The corresponding quinones appear to be the first stages of oxidation [Astre (5), Eller (*loc. cit.*)]. There is furthermore evidence that, in the case of the para-compounds (hydroquinone or para-benzoquinone) oxidation to hydroxyquinone (2-hydroxy, 1, 4-para-benzoquinone) is involved as an intermediate step. Experiments which we have conducted with this unstable compound, first prepared by Willstätter (64), support Eller's contention that it is an intermediate common to the oxidation of the several ortho- and para-phenols or quinones. However, the extreme lability of the ortho-benzoquinone [(Willstätter (62, 63)] makes very probable the assumption that the ortho-quinoid structure is an important factor in the later stages of oxidation. The condensation process leading to the dark-colored amorphous products may then involve ring rupture between the two adjacent quinone carbons and immediate re-condensation of the resulting open-chain intermediate, through its highly reactive terminal groups. Whether such a process is an intermediate in the formation of the "humus"-like products, whether a condensation of intact molecules is involved, or whether both take place is still an open question. Eller postulates the condensation of hydroxyquinone units; and it is well known that compounds of the diphenol type are formed under oxidizing conditions. Investigations still in progress by Raper and his co-workers (35, 48, 52, 53) on the oxidation of tyrosine and certain phenols by tyrosinase offer promise of shedding considerable light on the mechanism of the reactions involved in these later stages of phenol oxidation. In the case of the more complex phenols, such as pyrogallol, analogous reactions may be assumed to take place, although here the conditions are more complex.

As in the case of the carbohydrate reactions previously discussed, it has so far been impossible to isolate any specific intermediates at the stages where the dark-colored products appear. Under the conditions in which these reactions have been studied, the process of condensation proceeds

with great rapidity, leading to a complex mixture of products of varying degrees of condensation, solubility or dispersibility, and varying empirical composition.

Mention should also be made of another reaction product of "humus"-like nature, related to the substances formed from phenols, namely, the so-called "melanin" produced from tyrosine in the presence of tyrosinase. This reaction has been rather widely studied by a number of investigators, among them Bertrand (8, 9), Abderhalden and his co-workers (1, 2), and particularly Raper (52, 53), McCance (48) and Happold and Raper (35). The latter studies by Raper and his collaborators, which are still in progress, offer promise of greatly extending our knowledge of the mechanism of tyrosine oxidation as well as of the oxidation of phenols in general.

#### HUMUS-LIKE SUBSTANCES FORMED BY THE REACTION OF AMINO-ACIDS AND OTHER AMINO COMPOUNDS WITH CARBOHYDRATES OR ALDEHYDES

A third type of reaction leading to "humus"-like substances involves the reaction of amino acids, urea, etc., with sugars, furan aldehydes, etc. Maillard (40) described the formation of such "humic" substances by the reaction of glycine and other amino acids with glucose and other sugars in concentrated aqueous solution. Roxas (55) and Udransky (60) have studied similar reactions; and Gortner and his co-workers (14, 27, 28, 29, 30, 31, 32) as well as Dowell and Menaul (17) have attacked a related problem in the study of the formation of "humin" in the acid digestion of protein material in feedingstuffs for determination of amino-nitrogen by the Van Slyke method.

Rather extensive experiments and observations in this laboratory lead to the conclusion that this type of reaction involves the formation from the sugar or other carbohydrate material of a furan aldehyde (furfural or hydroxymethylfurfural) which, in turn, condenses through its aldehyde group with the amino group of the amino acid (amide group of urea, etc.), in a condensation of the "aldime" type, accompanied by decarboxylation in the case of carboxy compounds. This is then followed by a more profound condensation, probably involving ring rupture, as in the case of the furan aldehydes alone, resulting in the dark colored amorphous products.

These products contain nitrogen in rather uniform proportions, depending upon the composition of the reacting substances, and this nitrogen is tenaciously retained as a part of the complex. It is not separated even on rigorous chemical treatment and does not appear to be retained merely by adsorption or occlusion. In superficial properties the substances resemble those formed from carbohydrates alone or from phenols, exhibiting similar variations in solubility or dispersibility, etc.

## DISCUSSION

There are many other reactions known to lead to amorphous or resinous condensation products with properties similar to the "humus" substances; but those just cited represent typical examples involving compounds which occur more or less abundantly as plant constituents or are readily formed by degradation of such constituents. For instance the celluloses, as well as pentosans, starches, gums, or even the simple sugars, compose a large share of plant remains. Protein substances yielding amino acids are likewise plant constituents of widespread occurrence and are also important components of the remains of animal life and of microorganisms. Aromatic compounds of phenolic or quinone nature occur as constituents or are readily formed from more complex constituents, e.g. glucosides, resins, pigments, tannins, etc. Finally consideration must be given to lignin, next to cellulose probably the largest component of plant *débris* and, moreover, one of the most resistant to decomposition. The constitution of the lignins has not as yet been thoroughly elucidated, but there is evidence indicating a complex yielding, on decomposition, compounds of phenolic and furan aldehyde character. Hence, lignin may contribute to "humus" formation through any of the processes mentioned above.

All of the above-mentioned substances are not only known to gain access to soils through plant remains, but a number of representative individuals have been actually isolated from soils. A considerable proportion of such plant constituents is consumed as a source of energy by the soil microorganisms and is thus eliminated as a source of direct "humus" formation. However, there is still insufficient evidence to conclude that this applies to the total of such plant residues; it is probably a variable quantity under soil conditions. Furthermore, the microorganism can be considered as an intermediate in the general process, the remains of its structures and its metabolic products constituting stages in the series of reactions involved in the "humification" process.

Consideration of the above examples of reactions yielding "humus"-like material under controlled conditions indicates that any one of them or of others more or less related may play a rôle in the formation of "humus" under soil conditions. Naturally in the latter case the reactions taking place represent degrees of velocity and complexity differing widely from those of the laboratory. In place of vigorous reagents, high concentrations and rapid velocities we must assume the effects of high surface activity of soil particles, inorganic catalysts and highly potent biological factors, which, however, are capable of leading to the same end-products. It is conceivable that under certain conditions, physical, chemical, or biological, favorable to one type of reaction, or where the plant or animal remains gaining access to the soil are predominantly of one type, one course of reaction may predominate; under other conditions another

course may play a major rôle. All these reactions remain as possible contributors.

It is not within the scope of this paper to discuss the question of the theoretical and experimental evidence in regard to the actual precursors of "humus" substances in the soil or in regard to the nature of the actual processes there involved. The literature offers considerable discussion, sometimes of a controversial nature, as to whether cellulose or lignin substances, or both, are the main sources of humus formation [Eller (*loc. cit.*), Marcusson (*loc. cit.*), Waksman (61), Fischer and Schrader (25, 26), and others]. It must be remarked, however, that there has been some tendency to draw conclusions or make generalizations on the basis of evidence from too restricted a field. The accumulated data are as yet too meager to warrant conclusions too widespread in scope.

We merely seek to emphasize that, in view of the wide variety of these laboratory reactions yielding "humus"-like substances, we must conceive of the situation under natural soil conditions as extremely complicated, even from a chemical point of view alone, and ignoring a multitude of biological factors. The "humus" complex as found in the soil can be considered as representing an accumulation of the more resistant products, in varying degrees of chemical condensation, of a number of reactions as illustrated, involving a wide variety of parent substances.

The soil organic matter as a whole consists of a complex mixture of these "humus" substances and of greater or less quantities of the intermediates and by-products of the humifying reactions, as well as of other compounds not directly involved in the humification process. The latter consist of plant or animal constituents, or their derivatives, and products elaborated by microorganisms. The concentration of any one member of this soil organic complex at any one time will be a function of the equilibrium then existing between processes leading to its formation and processes leading to its decomposition. All may to a greater or less extent be combined physically or chemically with the inorganic soil constituents.

It is such a formidable complex which must represent at any given time the soil organic matter. It involves no static condition, but a dynamic equilibrium constantly shifting with the supply of raw material, alteration of the biological population of the soil and alteration in the physical or chemical conditions imposed by cultural methods or fertilizer application.

#### LITERATURE CITED

- (1) Abderhalden, E., and Guggenheim, M. 1907-1908. Hoppe-Seyler's *Ztschr. Physiol. Chem.* 54: 331; *ibid.* 57: 329.
- (2) ———, and Sickel, H. 1923. *Fermentforsch.* 7: 85.
- (3) Adler, O. 1923. *Biochem. Ztschr.* 137: 201; *ibid.* 141: 304.
- (4) ———, and Wiechowksi, W. 1922. *Ber. Deut. Chem. Gesell.* 55: 3030.
- (5) Astre, C. 1895. *Compt. Rend. Acad. Sci. [Paris]* 121: 326, 550, 559.
- (6) Beckley, V. A. 1921. *Jour. Agr. Sci. [England]* 11: 69.

- (7) Berthelot, M., and André, G. 1892. *Ann. Chim. et Phys.* [6] 25: 364, 403, 420.
- (8) Bertrand, G. 1896. *Compt. Rend. Acad. Sci. [Paris]* 122: 1215.
- (9) ———. 1908. *Ibid.* 146: 304.
- (10) Bottomley, W. B. 1915. *Biochem. Jour.* 9: 260.
- (11) Bouchardet, A. 1835. *Jour. Pharm.* 21: 627.
- (12) Boullay, P. 1830. *Ann. Chim. et Phys.* [2] 43: 273.
- (13) Braconnot, H. 1819. *Ibid.* 12: 172.
- (14) Burr, G. O., and Gortner, R. A. 1924. *Jour. Amer. Chem. Soc.* 46: 1224.
- (15) Conrad, M., and Guthzeit, M. 1885. *Ber. Deut. Chem. Gesell.* 18: 439, 2905.
- (16) ———, ———. 1886. *Ibid.* 19: 2569, 2575, 2844.
- (17) Dowell, C. T., and Menaul, P. 1919. *Jour. Biol. Chem.* 40: 131.
- (18) Düll, G. 1895. *Chem. Ztg.* 19: 166, 216.
- (19) Eller, W. 1921–1922. *Brennstoffchemie* 2: 129; *ibid.* 3: 49, 55.
- (20) ———. 1923. *Liebigs Ann. Chem.* 431: 133.
- (21) ———, and Koch, K. 1920. *Ber. Deut. Chem. Gesell.* 53: 1469.
- (22) ———, Meyer, H., and Saenger, H. 1923. *Liebigs Ann. Chem.* 431: 162.
- (23) ———, Herdieckerhoff, E., and Saenger, H. 1923. *Ibid.* 177.
- (24) Fenton, H. J. H., and Gostling, M. 1899, 1901. *Jour. Chem. Soc. [London]* 75: 423; *ibid.* 79: 361.
- (25) Fischer, F., and Schrader, H. 1921. *Brennstoffchemie* 2: 37.
- (26) ———, ———. 1922. *Ibid.* 3: 65, 341.
- (27) Gortner, R. A. 1916. *Jour. Biol. Chem.* 26: 177.
- (28) ———. 1919. *Science* 48: 122.
- (29) ———, and Blish, 1915. *Jour. Amer. Chem. Soc.* 37: 1630.
- (30) ———, and Holm, G. E. 1917. *Ibid.* 39: 2477.
- (31) ———, ———. 1920. *Ibid.* 42: 632, 2378.
- (32) ———, and Norris, E. R. 1923. *Ibid.* 45: 550.
- (33) Grote, A. v. and Tollens, B. 1875. *Liebigs Ann. Chem.* 175: 181.
- (34) ———, ———. 1877. *Ber. Deut. Chem. Gesell.* 10: 1440.
- (35) Happold, F. C., and Raper, H. S. 1925. *Biochem. Jour.* 19: 92.
- (36) Hibbert, H., and Hill, H. S. 1923. *Jour. Amer. Chem. Soc.* 45: 176.
- (37) Hofmann, F. 1920. *Brennstoffchemie* 1: 2.
- (38) Hoppe-Seyler, F. 1889. *Hoppe-Seyler's Ztschr. Physiol. Chem.* 13: 66.
- (39) Kiermayer, J. 1895. *Chem. Ztg.* 19: 1003.
- (40) Maillard, L. C. 1913. *Genèse des matières protéiques et des matières humiques.* Paris. Masson et Cie. Chaps. 18, 19, 20, 21.
- (41) Malaguti, S. 1836. *Liebigs Ann. Chem.* 17: 52–67.
- (42) Marcusson, J. 1918. *Ztschr. Angew. Chem.* 31: (I) 237.
- (43) ———. 1921. *Ber. Deut. Chem. Gesell.* 54: 542.
- (44) ———. 1921. *Ztschr. Angew. Chem.* 34: 437.
- (45) ———. 1922. *Ibid.* 35: 165.
- (46) ———. 1922. *Mitt. Materialprüf.* 40: 245.
- (47) ———. 1923. *Ztschr. Angew. Chem.* 36: 42.
- (48) McCance, R. A. 1926. *Biochem. Jour.* 19: 1022.
- (49) Mulder, G. J. 1840. *Jour. Prakt. Chem.* 21: 303; *Liebigs Ann. Chem.* 36: 243.
- (50) Péligot, E. 1838. *Ann. Chim. et Phys.* [2] 67: 113; *ibid.* 73: 208.
- (51) Pummerer, R., and Gump, W. 1923. *Ber. Deut. Chem. Gesell.* 56: 999.
- (52) Raper, H. S. 1926. *Biochem. Jour.* 20: 735; *ibid.* 21: 89.
- (53) ———, and Wormald, A. 1923. *Ibid.* 17: 454.
- (54) Robertson, R. A., Irvine, J. C., and Dobson, M. E. 1907. *Biochem. Jour.* 2: 458.
- (55) Roxas, M. L. 1916. *Jour. Biol. Chem.* 27: 71.
- (56) Sestini, F. 1880. *Gazz. Chim. Ital.* 10: 121.
- (57) Stein, W. 1839. *Liebigs Ann. Chem.* 30: 84.

- (58) Stoltzenberg, H., and Stoltezenberg-Bergius, M. 1920. Hoppe-Seyler's Ztschr. Physiol. Chem. 111: 1.
- (59) Trousov, A. 1914-1915. Selsk. Khoz. i. Liesov. 246: 233; *ibid.* 248: 409; Bull. Agr. Intell. 6: No. 4.
- (60) Udransky, L. v. 1887-1888. Hoppe-Seyler's Ztschr. Physiol. Chem. 11: 537; *ibid.* 12: 33.
- (61) Waksman, S. A. 1926. Soil Sci. 22: 123, 221, 323, 421.
- (62) Willstätter, R., and Pfannenstiehl, A. 1904. Ber. Deut. Chem. Gesell. 37: 4744.
- (63) ———, and Müller, H. E. 1911. *Ibid.* 44: 2171.
- (64) ———, ———. 1911. *Ibid.* 2180.

# NON-HUMUS CONSTITUENTS OF THE HUMUS EXTRACT

E. C. SHOREY

*United States Department of Agriculture, U. S. A.*

When a soil is treated with a dilute solution of alkali, ammonium, sodium or potassium hydroxide there results a dispersion of a portion of the dark colored organic soil colloids and there is obtained what is usually known as a humus extract.

This extract which can be separated from the soil by filtration is made up of a portion of the organic colloids dispersed to the dimensions capable of passing through ordinary filtering media such as filter paper, and also a solution of other organic constituents that occur in soils in relatively small quantities and capable of yielding a true solution in water, or forming a soluble compound with the alkali.

This alkaline humus extract is usually colored, dark brown in most cases, but light brown to yellow in the case of very sandy soils containing little organic matter.

When this colored extract is made slightly acid with a mineral acid there is immediate flocculation of a large proportion of the colored organic colloid and by filtration there can be effected a partial separation of this colloid from the other compounds soluble in the acid medium. The filtrate in this case is always colored and still contains some of the dispersed colloid which can be still further separated by addition of a soluble salt, sodium chloride or sulfate to saturation (salting out).

The organic compounds discussed in this paper are those found in the acid filtrate.

The quantity of any of the organic compounds that have been obtained from this acid filtrate has usually been quite small, in some cases too small for identification. A further quantity can generally be obtained by dispersing the humus precipitate again in alkali acidifying and filtering as in the first procedure.

The methods by which compounds have been obtained are two (1) Shaking the acid filtrate with an immiscible solvent—usually ether (2) Precipitation with metallic salts—usually mercuric sulfate, silver nitrate or sulfate, or lead acetate. Mercuric sulfate in sulfuric acid solution precipitates the colloid colored material and a number of crystallizable organic compounds from the acid filtrate. Lead and silver salts have been used only after the acid filtrate has been neutralized and filtered. These metallic precipitates have usually been decomposed by hydrogen

sulfide and the compounds so obtained separated by reprecipitation or treatment with suitable solvents.

When the acid filtrate is neutralized there is usually obtained a precipitate of alumina and other inorganic bases in combination with some of the organic constituents present. For the most part the organic constituents so precipitated are the same in character as those contained in the original humus precipitate. They can be separated from the alumina and other bases by suitable treatment and are generally dark colored and colloid in character.

As a rule the organic compounds that have been obtained in the manner indicated cannot be obtained from a soil by direct extraction with water or organic solvents, apparently for the reason that they are held by the colloidal humus and cannot be obtained in water or other solution until this colloidal material has been dispersed.

Nearly all classes of organic compounds are represented in those isolated and identified by the methods referred to. A list follows with reference to the publications in which details of such isolation and identification have been described. Picoline carboxylic acid (1, 2, 3); dihydroxystearic acid (2, 4); trihydroxystearic acid (5); hydroxy behenic acid (5) (phellenic acid); oxalic acid (6); benzoic acid (7), acrylic acid (6), saccharic acid (6), succinic acid (6); p. hydroxy benzoic acid (7); m. hydroxytoluic acid (6), oleic acid (5); and  $\alpha$ -crotonic acid (9); cyanuric acid (10, 11, 12); creatinine (13, 14); histidine (15, 16), arginine (15, 16), lysine (6); xanthine (15, 17), hypoxanthine (15, 17), adenine (6), cytosine (15, 17), mannite (6), rhamnose (6), nucleic acid (6), salicylic aldehyde (6), vanillin (7), trithiobenzaldehyde (6), choline (6).

The quantity of any compound obtained from a soil by isolation from the humus extract has usually been small often only a few milligrams from 100 pounds of soil, and stated as percentage appears very insignificant. Calculated to pounds per acre, foot, or six inches, however, it is apparent that in most cases the quantity of non-humus constituents is of the same order of magnitude as are the plant nutrients contained in an average fertilizer application.

While, as has been stated, the quantity of any single organic compound usually obtained from a soil is relatively small a few pounds per acre there have been cases where it was quite large at the rate of several hundred pounds or even several thousand pounds per acre foot. This has been the case with oxalic acid, mannite, benzoic acid, hydroxytoluic acid and cyanuric acid.

The distribution of the compounds found has been variable. Some such as benzoic acid, hydroxytoluic acid, oxalic acid and trithiobenzaldehyde have been found in but one or two soils in each case. Others such as cyanuric acid and the hydroxystearic acids have been found in soils of very diverse character and from widely separated locations.



The source of these non-humus soil compounds is largely a matter of conjecture. Where a compound is known to be a plant constituent it is perhaps fair to assume that it has been added to the soil in plant remains and has undergone no change. In the great majority of cases, however, there is nothing to indicate whether the compound is an unchanged plant constituent, the product of the activity of microorganisms, or a part of the remains of microorganisms.

This situation is the result of our dearth of knowledge both of plant constituents and of the products of the growth of microorganisms. With the exception of organic constituents of economic importance our knowledge of the organic composition of even our common crop plants is very meager and we have as a result a very imperfect knowledge of what compounds are added to soils in crop remains, weeds and green manure.

In the case of microorganisms except for those of pathogenic or industrial importance there is little knowledge of the character of the compounds resulting from their growth, multiplication, death and decay.

For these reasons we seldom know in the case of any organic compound what its fate will be when added to a soil. It is no doubt true that the final result of the decomposition of organic compounds in a soil is carbon dioxide, water and nitrogen or nitrates but these end products are reached only through stages and these vary with the compound and its environment.

The question whether the organic compounds isolated from soils by the methods briefly outlined are present in the soil as such or whether they may have been formed from some other compound by the treatments involved is one that has been raised from time to time and is one that any investigator should keep in mind in presenting results of such work or seeking to fix the rôle that they may play in the soil. Any sweeping statement that these compounds have for the most part been formed from other compounds by the methods used, could be made only where the chemical properties of the compounds and of the methods by which they may be formed have been ignored. If such a statement were true it would apply equally to our knowledge of the chemical constituents of plant and animal tissues, in the investigation of which similar methods have been used.

A survey of the possibilities in this connection presents the following conclusions: Many of the acids isolated by these methods probably are present in soils as salts of iron aluminum, calcium, etc. Others especially the hydroxy acids may be present in organic combination and the acid set free by the action of dilute alkali, although in these cases it is easily shown that such combinations are not readily broken down by dilute alkali at room temperature.

Nucleic acid is exceedingly resistant to change by dilute alkali; in fact it has always been prepared from other sources just as it has been obtained

from soils. The only known natural source of the purine and pyrimidine bases is nucleic acids, or nucleic acid and they cannot be obtained from such sources by any such mild treatment as that to which soil has been subjected in these researches.

Cyanuric acid can be obtained from cyamelid by treating with dilute alkali, in fact the isolation of cyanuric acid might be taken as evidence of the presence of cyamelid in soils. Cyamelid is a polymer of cyanuric acid so that their chemical and biological relations are not very different in either case.

No raw plant or micro biological material is known that will yield such compounds as mannite, vanillin, or salicylic aldehyde by treatment with dilute alkali at room temperature.

In short, consideration of the properties and relationships of the isolated compounds and of the methods employed does not justify the conclusion that these soil compounds have been formed from other material except where they may have been obtained from closely related compounds such as an acid from a salt or an ester.

For the most part the non-humus organic compounds that have been isolated from the humus extract of soils may be regarded as representing stages in the decomposition of the more complex constituents of plants and animals, and as such are constantly changing both in character and quantity.

The rôle that such compounds may play in soil fertility or infertility is one that our present lack of knowledge of all the factors involved makes it impossible to fix. It is quite certain, however, that they are important factors that should receive more consideration than has been accorded. Any organic compound that is soluble even slightly in the soil moisture must have an effect on plants growing in the soil, and on the activity of microorganisms, both as regards the nature of their products and the rate of increase.

Furthermore such compounds being reactive with inorganic compounds tend to alter or modify the general chemical changes constantly going on in soils.

#### LITERATURE CITED

- (1) Shorey, E. C. 1906. Report of Chemist. Report of Hawaii Experiment Station 1906.
- (2) Schreiner, O., and Shorey, E. C. The isolation of harmful substances from soils. U. S. Dept. of Agr., Bur. Soils Bul. No. 53.
- (3) ———, ———. 1908. The isolation of picoline carboxylic acid from soils and its relation to soil fertility. Jour. Amer. Chem. Soc. Vol. 30.
- (4) ———, ———. 1908. The isolation of dihydroxystearic acid from soils. Ibid. Vol. 30.
- (5) Shorey, E. C. Unpublished data.
- (6) ———. 1913. Some organic soil constituents. U. S. Dept. Agr. Bur. Soils Bul. No. 88.

- (7) ———. 1914. The presence of some benzene derivatives in soils. *Jour. Agr. Research* [U. S.] Vol. 5.
- (8) Walters, E. H. 1917. The isolation of p. hydroxbenzoic acid from soils. *Jour. Amer. Chem. Soc.* Vol. 39.
- (9) ———, and Wise, L. E. 1916.  $\alpha$ -Crotonic acid a soil constituent. *Jour. Agr. Research* [U. S.] Vol. 6.
- (10) Shorey, E. C., and Walters, E. H. 1914. A nitrogenous soil constituent tetracarbonimid. *Ibid.* Vol. 2.
- (11) Wise, L. E., ——— 1917. Isolation of cyanuric acid from soil. *Ibid.* Vol. 10.
- (12) Walters, E. H., and Wise, L. E. 1917. The identity of cyanuric acid with so-called "tetracarbonimid." *Jour. Amer. Chem. Soc.* Vol. 39.
- (13) Shorey, E. C., Sullivan, M. X., and Skinner, J. J. 1911. A beneficial organic constituent. Creatinine. U. S. Dept. Agr. Bur. Soils Bul. No. 83.
- (14) Shorey, E. C. 1912. The isolation of creatinine from soils. *Jour. Amer. Chem. Soc.* Vol. 34.
- (15) Schreiner, O., and Shorey, E. C. 1910. Chemical nature of soil organic matter. U. S. Dept. Agr. Bur. Soils. Bul. No. 74.
- (16) ———, ———. 1910. The presence of arginine and histidine in soils. *Jour. Biol. Chem.* Vol. 8.
- (17) ———, ———. 1910. Pyrimidine derivatives and purine bases in soils. *Ibid.* Vol. 8.

# THE CARBON-NITROGEN RATIO AND MICROBIOLOGICAL INVESTIGATION OF THE SOIL IN RICE FIELDS

A. ITANO <sup>1</sup>

*Ohara Institute of Agricultural Research, Japan -*

## INTRODUCTION

The lack of information in regard to the subject and the importance attached to it, prompted the author to undertake the following investigation. Only a short report, preliminary in nature will be given here since the investigation was started in June, 1925, just before planting the rice and the second year is in progress at present.

From the physico-chemical nature of the rice field and also from the nature of manure used in Japan, it is anticipated that some interesting as well as valuable information on the subject should be found.

The experimental plot is a portion of the field which has been under cultivation for a few hundred years and the soil has the following composition.

TABLE I.—Average analysis of the soil in experimental field \*

Composition	Rice field (Alluvial, sandy loam)	Dry farm (Alluvial loam)
	per cent	per cent
Moisture	2.640	2.770
Loss on ignition	6.570	7.370
Total humus	1.780	1.380
Total nitrogen	0.287	0.165
Cl	0.216	0.011
FeO	0.102	0.451
Fe <sub>2</sub> O <sub>3</sub>	3.750	2.180
Al <sub>2</sub> O <sub>3</sub>	4.920	1.070
Mn <sub>2</sub> O <sub>3</sub>	0.300	0.320
CaO	0.605	0.500
SO <sub>3</sub>	0.036	0.041
P <sub>2</sub> O <sub>5</sub>	0.089	0.161
K <sub>2</sub> O	0.257	0.307
Na <sub>2</sub> O	0.480	0.491
SiO <sub>2</sub> sol. in HCl	0.501	0.428
Do Na <sub>2</sub> CO <sub>3</sub>	9.820	7.830
Do H <sub>2</sub> SO <sub>4</sub>	2.446	2.045
Al <sub>2</sub> O <sub>3</sub> Do Do	2.188	1.925
Fe <sub>2</sub> O <sub>3</sub> Do Do	0.888	1.013

\* S. Osugi and N. Soyama, *Nogaku-Kaiho*, 233, 115, 1922.

<sup>1</sup> Assisted by Satiyo Arakawa.

## EXPERIMENTAL

A portion of the experimental field was sectioned off, by the brick wall, into forty-four sections of one Tsubo <sup>1</sup> each. Each four of these sections constitute a plot. The results are reported per Tsubo, taking an average of the four small sections located on each side of a row.

Previous to the investigation, six representative plots were examined for their Carbon <sup>2</sup>-nitrogen <sup>3</sup> ratio and hydrogen ion concentration,<sup>4</sup> and the results are shown in the following table:

*Table 2.—Carbon-nitrogen ratio and hydrogen ion concentration*

Plots	Carbon	Nitrogen	C:N	Reaction
	per cent	per cent		pH
1	1.671	0.127	13.2	6.71
3	1.553	0.130	11.9	6.56
5	1.556	0.125	12.4	6.69
7	1.558	0.123	12.7	7.19
9	1.905	0.117	15.4	7.00
11	1.304	0.112	11.6	6.88
Mean	1.575	0.122	12.9	6.84

Examining the above table in the light of some results reported by the authors in foreign countries, the soil here has a wider C:N ratio than the average which is about 10:1. However, some investigators, for example Brown and Oneal <sup>5</sup> found that in a Carrington loam, the ratio was from 12:1 to 13:1 which is very similar to the results noted above.

Each plot was treated differently with or without the addition of various combinations of fertilizers which are commonly used on the farm in this section of Japan, as shown below:

*TABLE 3.—Quantity and quality of fertilizers applied to each plot*

Plots	Compost	Soybean cake	Cotton seed cake	Night soil	Acid-phosphates	Ash	Dry Genge	Straw	Green red clover
1	3,750.0 *	187.5	123.8	1,248.8	86.3	150.0	48.8		
2									
3								13,650.0	
4								16,500.0	
5								23,100.0	
6	1,875.0							13,650.0	1,875.0
7								16,500.0	1,875.0
8									
9									
10									1,875.0
11									3,750.0

\* Data in grams per Tsubo or 3.31 square meters.

<sup>1</sup> Tsubo = 3.31 square meters.

<sup>2</sup> Determined by electrical combustion method as usual.

<sup>3</sup> Do Kjeldahl method, carried out by K. Hosoda.

<sup>4</sup> Do electrometric gas chain method.

<sup>5</sup> Brown, P. E., and Oneal, A.M., Research Bulletin 75, Iowa Agr. Exp't Station.

As Table 3 indicates, Plot 2 received a complete fertilizer as generally is applied here, and Plots 3, 4, and 5 received the straw exclusively in various amounts to determine its specific effect, if possible.

The carbon-nitrogen ratio of the humus forming materials used as the fertilizer, is given in Table 4.

TABLE 4.—The carbon-nitrogen ratio of humus forming materials used

Names	Moisture content	Carbon	Nitrogen	C:N
		per cent	per cent	
Compost	76.35	22.41	1.77	12.7
Soybean cake	10.53	50.03	9.25	5.4
Cotton seed oil cake	11.88	48.16	7.35	6.6
Genge *	9.27	46.37	2.68	17.3
Straw (barley)	15.93	46.49	0.53	87.7
Red clover	79.85	45.30	3.99	11.4

\* *Astragalus sinicus*.

The total carbon and nitrogen, added to each plot, were determined and the carbon-nitrogen ratio was calculated as shown in Table 5.

TABLE 5.—The carbon-nitrogen ratio added to each plot

Plots	Per cent determined		C:N
	Carbon	Nitrogen	
1			13.2
2	0.14	0.022	6.4
3	2.13	0.024	88.8
4	2.58	0.029	89.0
5	3.61	0.041	88.0
6	2.20	0.026	84.6
7	2.65	0.036	73.6
8	0.04	0.003	13.3
9	0.11	0.009	12.2
10	0.14	0.012	11.7
11			11.6

As shown in Table 5, Plots 3, 4, 5, 6 and 7 were provided with very wide carbon-nitrogen ratio while in case of Plot 2, the ratio was 6.4.

The rice, Ehime Shinriki variety, was planted on July 3, 1925. This variety was used not for its best quality but, in fact, it was the only available variety on hand at the time. The daily observations were made as to the growth, particularly color of leaves, gas production from the soil and color of water.

The crop was harvested on November 2, 1925 and treated as usual. The results are given in Table 6.

Table 6.—Amount of crop harvested in 1925

Plots	Rice in Hask (Kilogram per Tan. <sup>a</sup> )	Straw (Kilogram per Tan.)
1	Not planted	
2	569.9	1,143.9
3	397.6	733.2
4	386.1	714.0
5	321.0	604.5
6	439.3	832.1
7	360.9	704.7
8	477.5	834.9
9	548.6	999.1
10	523.5	1,060.4
11	455.2	853.7

<sup>a</sup> Tan =  $\frac{1}{4}$  of an acre.

The above results indicate that the control which received a complete fertilizer gave the best results although it had only a 20 per cent increase over the unfertilized plot. In Plots 3, 4 and 5 to which the barley straw was applied, the amount of crop was in inverse ratio to the amount of straw applied. Plots 6 and 7 where the red clover was applied gave a similar result. It seems to indicate that the application of fresh straw is detrimental to the crop as found by the others. Plots 8, 9 and 10 where the red clover and the compost were applied, gave a fairly good crop.

After the harvest, the second analyses of the soil in each plot were made for the total carbon and nitrogen, and hydrogen ion concentration, and the results are given in Table 7.

Table 7.—Carbon-nitrogen ratio and hydrogen ion concentration

Plots	Loss on ignition	C	N	C:N	Reaction <sup>a</sup>
	per cent	per cent	per cent		pH
1	6.81	1.759	0.240	7.3	6.94
2	6.87	1.730	0.266	6.7	6.82
3	6.84	1.720	0.273	6.3	7.17
4	6.87	1.678	0.273	6.1	7.11
5	6.78	1.700	0.258	6.6	6.83
6	6.71	1.614	0.238	6.8	6.81
7	6.78	1.660	0.199	8.3	7.06
8	7.06	1.669	0.190	8.8	7.04
9	7.11	1.796	0.181	9.9	6.84
10	6.57	1.659	0.154	10.8	7.08
11	5.96	1.522	0.159	9.6	6.85

<sup>a</sup> Determined by the Quinhydrone method.

The above table indicates that the total organic matter, determined by ignition became somewhat the same in all the plots, and the C:N ratio became much narrower. It is noteworthy that the nitrogen content in Plots 1 to 6 inclusive, is higher than that of the other plots.

The correlation between the C:N ratio and the crop harvested is shown in Table 8.

*TABLE 8.—Correlation between the C:N ratio and crop harvested*

Order by		Crop in kg.	C:N Ratio	Comparative per cent, Plot 2 as 100
Crop	Plots			
1	2	569.9	6.7	100
2	9	548.6	9.9	96
3	10	523.5	10.9	92
4	8	477.5	8.8	84
5	11	455.2	9.6	80
6	6	439.3	6.8	77
7	3	397.6	6.3	70
8	4	386.1	6.1	67
9	7	360.9	8.3	63
10	5	321.0	6.6	56

From the results obtained, it is difficult to find any definite correlation between the crop productivity and C:N ratio. But on an average, the wider ratio gave better crops. •

These results will be considered carefully in detail later, when the microbiological analyses of the soil in these plots is completed.



# L'OXYDATION MICROBIENNE DU SOUFRE DANS SES RAPPORTS AVEC L'ÉVOLUTION DE LA MATIÈRE AZOTÉE DANS LE SOL

G. GUITTONNEAU

*Institut des Recherches Agronomiques, Paris, France*

Nous savons depuis longtemps déjà que le soufre élémentaire et ses composés incomplètement oxydés (hyposulfites, thionates) sont transformés en sulfates par certaines espèces microbiennes qui puisent dans les oxydations mêmes qu'elles produisent l'énergie nécessaire à leur développement (1, 12, 13, 16).

De tels microorganismes autotrophes ont été isolés du sol ou de composts riches en soufre (7, 15, 20, 21, 22) et ils peuvent, sans aucun doute, jouer un rôle important dans la transformation biologique du  $\text{Sen H}_2\text{SO}_4$  (4) phénomène dont nous connaissons aujourd'hui tout l'intérêt agronomique (14, 15, 23).

Mais certaines espèces microbiennes hétérotrophes du sol sont elles aussi très actives vis à vis du soufre (5, 17) et il est infiniment probable qu'elles interviennent dans le métabolisme de cet élément, lorsque celui-ci est incorporé à une terre arable. Les modalités d'une telle intervention nous étant encore fort imparfaitement connues, j'ai voulu essayer de les préciser.

Dans ce but, et à la suite d'observations agricoles et viticoles qui seront ultérieurement publiées, j'ai été conduit à rechercher les rapports qui peuvent exister dans un sol riche en azote organique entre l'évolution de cet azote et l'oxydation microbienne du soufre.

En 1912, Boullanger et Dugardin (3) avaient déjà signalé les influences diverses que peut exercer la présence du soufre sur l'action des ammonisateurs, des ferments nitreux, des ferments nitriques ou des fixateurs d'azote. Vogel (19) a confirmé ou complété les observations de Boullanger et Dugardin. Mais ces savants ne se sont pas attachés à expliquer le mécanisme des actions favorables ou défavorables qu'ils observaient et ils n'ont pas suivi l'évolution du soufre au cours de leurs expériences.

C'est dans le but d'acquérir quelques unes des notions qui nous manquent à ce sujet que je me suis posé les deux problèmes suivants:

(1) Déterminer par quels mécanismes, directs ou indirects, le soufre incorporé à une terre riche en matière organique azotée s'y transforme en sulfates.

(2) Ces mécanismes étant connus, rechercher comment, dans leurs di-

verses phases, ils peuvent influencer l'ammonisation, la nitrification et la dénitrification.

Dans ma communication je n'aborderai que le premier de ces problèmes et je ne m'occuperai par conséquent que du soufre.

### ESPRIT, CONDITIONS GÉNÉRALES ET DIVISION DE CETTE ÉTUDE

Pour préciser les conditions de mes recherches, je me suis astreint à les faire toutes porter sur une même terre: celle du jardin de l'Institut national Agronomique de Paris. Ce choix n'a été motivé que par une raison de commodité personnelle et il peut être critiqué: comme la plupart des terres des jardins de Paris celle que j'ai étudiée est très riche en matières humiques et sa teneur en  $\text{CaSO}_4$  est excessive. Mais c'est une terre fertile et cela suffisait en somme à tous les besoins de mes démonstrations présentes et futures.

Pour que cette terre dont la réaction est voisine de la neutralité, ( $\text{pH} = 6,8$ ) ne s'acidifie jamais d'une manière sensible au cours de mes expériences, je lui ai toujours intimement incorporé du  $\text{CaCO}_3$  précipité, en excès par rapport aux doses de soufre employées.

Ma terre d'expérience étant ainsi définie, j'ai recherché comment sa flore microbienne réagissait sur le soufre précipité en présence et en absence de peptone (peptone Chapoteaut). A cet effet:

(a) J'ai tout d'abord suivi par l'analyse chimique l'évolution du soufre dans la terre elle même et cela aussi parfaitement qu'il était possible de la faire dans ce milieu complexe. J'ai ainsi trouvé que la transformation du soufre en sulfates dans un sol riche en matière organique azotée relève, pour une large part, d'un mécanisme complexe qui comporte plusieurs phases.

(b) J'ai déterminé les conditions les plus favorables à la réalisation des diverses phases de la transformation observée en cherchant à les faire apparaître dans des milieux liquides bien définis que j'ensemenciais avec de la délayure des terres d'expérience.

(c) Les espèces prédominantes dans les terres d'expérience ayant été isolées aux moments opportuns et les conditions les plus favorables au développement de ces espèces étant connues, il ne me restait qu'à reproduire en cultures pures ou associées les phénomènes que j'avais directement observés dans la terre.

Ce cadre général de mes recherches sera également celui de mon exposé.

### I. L'ÉVOLUTION DU SOUFRE DANS LA TERRE<sup>1</sup>

Dans toutes les expériences résumées ci-après, la terre passée au tamis Nr. 15 et rendue aussi homogène que possible a été divisée en 4 lots qui ont

<sup>1</sup> Toutes les études rapportées dans ce chapitre ont été faites en collaboration avec J. Keilling, préparateur à la Station Centrale de Microbiologie de Paris.

reçu respectivement par Kilo de terre sèche: Lot Nr. 1,  $\text{CaCO}_3$  de 20 à 30 g.; Lot Nr. 2,  $\text{CaCO}_3$  de 20 à 30 g., soufre précipité de 6 à 7 g.; Lot Nr. 3,  $\text{CaCO}_3$  de 20 à 30 g., soufre précipité de 6 à 7 g., peptone de 4 à 5 g.; Lot Nr. 4,  $\text{CaCO}_3$  de 20 à 30 g., peptone de 4 à 5 g. Ces différents produits ont été très intimement mélangés à la terre et celle-ci, abandonnée à la température du laboratoire, a été maintenue à un degré d'humidité de 16 à 17° et remuée de temps en temps. Dans quelques cas un lot Nr. 5 identique au lot Nr. 3 a été stérilisé 3 jours de suite pendant 1 heure à 130° maintenu à l'abri des poussières de l'air et arrosé aseptiquement avec de l'eau stérile.

Les résultats des analyses successives pratiquées sur les terres ainsi préparées seront examinés aux trois points de vue suivants: (1) La formation des sulfates aux dépens du soufre. (2) La solubilisation de soufre et la formation de composés du soufre intermédiaires entre le soufre élémentaire et les sulfates. (3) La formation des hyposulfites.

#### A. FORMATION DES SULFATES AUX DÉPENS DU SOUFRE

Mes recherches sur ce point n'ont fait que confirmer ce fait bien connu que tout le soufre incorporé à une terre, additionnée ou non d'une matière organique comme la peptone, finit par se transformer en sulfates. Le Tableau 1 montre la marche générale du phénomène global dans le cas qui nous intéresse ici.

TABLEAU 1.—Transformation du soufre en sulfates dans la terre. Le soufre des sulfates totaux est exprimé en milligrammes par Kilo de terre sèche. Les terres des lots Nr. 2 et Nr. 3 ont reçu 6 grammes de soufre par Kilo de terre sèche

Temps écoulé depuis le début de l'expérience, en jours	0	7	14	21	34	90	210	294	440
Lot Nr. 1	880	680	680	440	680	680	760	1120	1120
Lot Nr. 2	880	920	1640	2760	2800	2240	3120	4320	5880
Lot Nr. 3	880	880	1040	1040	1280	2120	4280	4960	5600

#### B. SOLUBILISATION DU SOUFRE—FORMATION DES COMPOSÉS DU SOUFRE INTERMÉDIAIRE ENTRE LE SOUFRE ÉLÉMENTAIRE ET LES SULFATES

Si au lieu d'étudier la formation des sulfates totaux (solubles et insolubles dans l'eau) on porte son attention sur la solubilisation du soufre et si on examine les extraits aqueux des terres d'expérience, préparés à la température ordinaire et rendus parfaitement limpides par filtrations, on constate ce qui suit:

(a) La solubilisation du soufre est beaucoup plus rapide que la formation des sulfates solubles.

(b) Toutes choses égales d'ailleurs, la vitesse de cette solubilisation du soufre est considérablement accrue par la présence de peptone.

(c) Au cours d'une même expérience, l'écart enregistré entre le chiffre qui exprime la richesse d'une terre en soufre soluble total et celui qui correspond au soufre de ses sulfates solubles va en diminuant avec le temps et finit par devenir nul.

(d) La solubilisation du soufre dans les terres d'expérience est due à l'action des microorganismes. En effet, cette solubilisation ne se produit pas si la terre, le  $\text{CaCO}_3$ , le S et la peptone mis en oeuvre ont été préalablement stérilisés et si le tout est tenu à l'abri des contaminations extérieures.

Ces diverses conclusions ressortent de l'examen des chiffres du Tableau 2.

TABLEAU 2.—*Soufre solubilisé dans la terre. Le soufre soluble est exprimé en milligrammes par Kilo de terre sèche*

Temps écoulé depuis le début de l'expérience, en jours	0	7	97	114	868*
(I)	(II)	(III)	(IV)	(V)	(VI)
Lot Nr. 1 Soufre des sulfates solubles	124	113		204	
Soufre soluble total	230	141		225	
Lot Nr. 2 Soufre des sulfates solubles	35	166		1017	1107
Soufre soluble total	217	397		1017	1107
Lot Nr. 3 Soufre des sulfates solubles	117	191	1103		1078
Soufre soluble total	261	1083	2348		1078
Lot Nr. 4 Soufre des sulfates solubles	95	110	267 <sup>b</sup>		
Soufre soluble total	190	181	402 <sup>b</sup>		
Lot Nr. 5 Soufre des sulfates solubles				88	
Soufre soluble total				176	

\* Les chiffres de la colonne VI et ceux des colonnes II, III, IV et V se rapportent à deux expériences de dates différentes mais réalisées dans des conditions rigoureusement identiques. Leur rapprochement montre la disparition des produits intermédiaires solubles.

<sup>b</sup> Dans cette terre très riche en sulfate de calcium la solubilité de ce sel est influencée par diverses manifestations de l'activité microbienne dans le sol. Cela explique les anomalies surprenantes à première vue que l'on relève dans le tableau ci-dessus.

Nous devons conclure de ce qui précède: (a) qu'entre le soufre élémentaire et le soufre totalement oxydé des sulfates le métabolisme microbien peut faire apparaître dans le sol des formes intermédiaires solubles du soufre qui finissent elles mêmes par se transformer en sulfates; (b) qu'une ammonisation active semble favoriser la formation de ces produits intermédiaires.

L'analyse chimique m'a en outre montré que parmi ces produits intermédiaires on ne constate jamais la présence des sulfures dans les conditions de mes recherches.

## C. FORMATION DES HYPOSULFITES

Par contre, au bout de quelques jours d'expérience et en opérant toujours sur les extraits aqueux des terres traitées, j'ai quelquefois pu déceler de faibles doses d'hyposulfites dans les lots Nr. 2; j'ai toujours trouvé ces composés dans les lots Nr. 3 et je n'ai jamais pu les caractériser ni dans les lots Nr. 1 ni dans les lots Nr. 4.

Mais les hyposulfites ainsi régulièrement trouvés dans les lots Nr. 3 ne représentaient jamais la totalité des produits intermédiaires signalés dans le paragraphe précédent. Si, d'ailleurs à divers expériences, on établit le rapport qui existe entre le soufre des hyposulfites et celui des autres produits intermédiaires on obtient des valeurs extrêmement variables. (Tableau 3.)

TABLEAU 3.—Rapport entre le soufre des hyposulfites et le soufre des produits intermédiaires solubles autres que les hyposulfites dans les terres des lots Nr. 3

Nr. de l'expérience	Nombre de jours écoulés depuis le début de l'expérience	Soufre des hyposulfites (en mg. par kg. de terre sèche)	Soufre des produits intermédiaires autres que les hyposulfites (en mg. par. kg. de terre sèche)	Rapport entre le soufre des hyposulfites et le soufre des autres produits intermédiaires
60	7	204	688	0.29
60	97	176	1069	0.16
65	8	138	605	0.22
65	22	68	1089	0.06
70	33	1520	397	4.00

Tout se passe donc comme si, entre le soufre élémentaire et les sulfates, il se formait une série de produits intermédiaires susceptibles de se transformer les uns dans les autres. Les hyposulfites ne constitueraient que l'un des termes de cette série, mais ce terme m'a semblé particulièrement intéressant pour trois raisons surtout: (a) Les hyposulfites représentent une forme relativement stable du soufre dans les milieux neutres ou alcalins; (b) Ces composés sont facilement transformés en sulfates par le travail de certaines espèces microbiennes; Au cours même de cette transformation peuvent apparaître d'autres produits intermédiaires se rattachant à la série thionique (Trautwein (18)).

## II. L'OXYDATION MICROBIENNE DU SOUFRE EN MILIEU LIQUIDE ET EN CULTURES IMPURES

Pour simplifier mes études et pour en éliminer l'influence de la dénitrification, j'ai préparé par tâtonnement une solution minérale (Solution nutritive II) ne contenant ni soufre ni azote, mais de constitution telle qu'

après addition sous une forme convenable des éléments nutritifs qui lui manquent elle puisse assurer le meilleur développement possible aux espèces microbiennes qui m'intéressaient. Voici la composition de cette solution:  $K_2HPO_4$ , 1 g.;  $MgCl_2$ , 0,2g.;  $CaCl_2$ , 0,2g.;  $Fe_2Cl$ , 0,01g.;  $MnCl_2$ , 0,005g.;  $ZnCl_2$ , 0,005g.;  $K_2SiO_3$ , 0,005g.; eau distillée q.s. pour 1000 cc.

Les milieux nutritifs préparés en partant de cette solution ont toujours été neutralisés par la soude en présence de phénolphtaléine comme indicateur (pH 7,6).

Dans la solution nutritive II peptonée, additionnée de soufre et stérilisée, puisensemencée avec de la délayure de terre des lots Nr. 3, l'évolution de soufre est sensiblement la même que dans la terre de ce lot (Tableau 4).

TABLEAU 4.—Cultures impures sur solution minérale peptonée à 0,5% et additionnée de 5% de soufre précipité. Les chiffres du tableau expriment le soufre solubilisé en milligrammes pour 100 centimètres cubes de culture

Temps écoulé depuis le début de l'expérience	12 jours	28 jours	54 jours
Soufre soluble total	48	108	134
Soufre des sulfates	0	5	19
Soufre des hyposulfites	48	101	11
Soufre non déterminé	0	2	104

En possession de la formule de cette solution nutritive je pouvais donc aborder l'étude des microorganismes isolés, dans les conditions les plus diverses, des terres des lots Nr. 3.

### III. L'OXYDATION DU SOUFRE SOUS L'ACTION D'ESPÈCES MICROBIENNES PURES TRAVAILLANT SEULES OU EN ASSOCIATION

Dans ce chapitre, je résumerai les résultats de mes recherches en examinant: (A) la solubilisation du soufre à l'état d'hyposulfites. (B) la transformation des hyposulfites en sulfates. (C) la transformation du soufre en sulfates par voie d'association microbienne.

#### A. LA SOLUBILISATION DU SOUFRE A L'ÉTAT D'HYPOSULFITES

J'ai montré (8, 10, 11) que, dans un milieu qui s'alcalinise ou qui reste neutre et dans des conditions de cultures rappelant les diverses phases de l'ammonisation, beaucoup de microorganismes ammonisateurs peuvent solubiliser le soufre à l'état d'hyposulfites.

Avec certaines bactéries et avec certains actinomycètes, les hyposulfites se forment en quantité importante dans des milieux nutritifs liquides préparés en ajoutant à la solution minérale II un aliment organique azoté convenable (de peptone, par exemple) et du soufre précipité. Il n'ap-

paraît alors en proportion appréciable aucun autre composé du soufre soluble que les hyposulfites. On constate en effet une concordance aussi parfaite que possible entre les deux chiffres obtenus en dosant le soufre: (a) l'état de  $S_2O_2$  par une liqueur d'iode 0.025 N; (b) à l'état de  $SO_3$  (dans  $BaSO_4$ ) après oxydation par l'hyprobromite et le brome (technique de Denigès (6) (voir Tableau 5)).

*TABLEAU 5.—Production d'hyposulfites aux dépens du soufre par quelques espèces microbiennes. Caractérisation des hyposulfites. Les cultures ont été faites sur solution minérale additionnée de 1% de peptone et de 5% de soufre. Les résultats sont exprimés en milligrammes de soufre pour 100 cc. de culture*

Microorganismes étudiés		Actino- mycès griseus	Bacillus migate- rium	Bacillus mycoides	Bacillus mesen- tericus	M.M.
Soufre solubilité	Dosé par l'iode à l'état d'hyposulfites	48	196	36	38	180
	Dosé en poids à l'état de sulfates après oxydation par le brome	50	198	38	40	186

D'autres bactéries que celles qui figurent dans le Tableau 5 ne solubilisent en culture pure que de faibles doses de soufre à l'état d'hyposulfites. Les déterminations quantitatives manquent alors de netteté, mais les cultures en association dont il sera question plus loin montreront tout l'intérêt que présente une telle solubilisation, quelle que soit l'intensité avec laquelle elle se manifeste en cultures pures.

Dans toutes les cultures liquides dont il vient d'être question je n'ai jamais vu se former de sulfates aux dépens du soufre. Il ne s'en est pas formé davantage lorsque j'ai placé les microorganismes étudiés dans les conditions les plus favorables aux oxydations biologiques maxima. On verra en effet dans le Tableau 6 les résultats que m'a fournis l'analyse du liquide obtenu en épuisant par l'eau chaude des cultures faites, en présence de soufre précipité, sur milieux gélosés à base de solution minérale II peptonée ou de bouillon de haricots peptoné et sucré.

Dans les diverses conditions expérimentales que j'ai réalisées les microorganismes étudiés (bactéries ammonisatrices, actinomycètes) n'ont donc jamais donné de sulfates aux dépens du soufre; ils ont presque toujours formé des hyposulfites et quelquefois en outre sur milieux solides surtout, des produits intermédiaires autres que les hyposulfites.

## B. TRANSFORMATION DES HYPOSULFITES EN SULFATES

Par divers artifices de technique, j'ai isolé (9) plusieurs types de bactéries capables de transformer les hyposulfites en sulfates. Je n'en ai retenu que trois.

TABLEAU 6.—*Production d'hyposulfites aux dépens du soufre sur milieux gélosés par divers microorganismes ammonisateurs. Les chiffres du tableau représentent des milliagrammes de soufre solubilisé dans une boîte de Roux contenant 50 cc. de milieu gélosé et 5 g. de soufre*

Microorganismes étudiés	M M		Actinomyces griseus		Bacillus Misenlericus		Bacillus megaterium		Bacillus mycoides	
	Solution minérale peptonée	Bouillon de haricots peptoné sucré	Solution minérale peptonée	Bouillon de haricots peptoné sucré	Solution minérale peptonée	Bouillon de haricots peptoné sucré	Solution minérale peptonée	Bouillon de haricots peptoné sucré	Solution minérale peptonée	Bouillon de haricots peptoné sucré
Milieu gélosé										
Soufre des sulfates	0	0	0	0	0	0	0	0	0	0
Soufre de $S_2O_3$ (iode)	65	32	41	13	39	23	39	0	23	34
Soufre de $SO_2$ après oxydation	78	32	44	14	54	30	47	0	26	50



OS<sub>3</sub> est une petite bactérie très voisine de *Thiobacillus thio parus* (Beijerinck (2)).

T<sub>2</sub> et b<sub>2</sub> poussent sur les milieux gélosés à base de décoctions végétales (touraillon, haricots) peptonées et sucrées.

Sur ces milieux T<sub>2</sub> forme des colonies d'un rose tirant sur le rouge et b<sub>2</sub> des colonies blanches qui se plissent et rappellent beaucoup celles de certains bacilles du groupe du *B. subtilis*. Mais b<sub>2</sub> diffère du *B. subtilis* type par plusieurs caractères.

Dans mes expériences, OS<sub>3</sub>, T<sub>2</sub> et b<sub>2</sub> n'ont jamais ammonié d'une manière appréciable la peptone que je leur offrais, comme aliment azoté, dans la solution minérale II. Dans un milieu ainsi constitué leur développement est d'ailleurs pénible. Les sels ammoniacaux organiques ou minéraux leur conviennent mieux que la peptone comme aliment azoté et, dans un milieu minéral ne renfermant pas d'autre matière organique que celles des impuretés apportées par les éléments constitutifs de ce milieu, elles transforment encore les hyposulfites en sulfates. Ces espèces ont donc au moins une tendance très marquée à l'autotrophisme.

Les bactéries OS<sub>3</sub> et T<sub>2</sub> peuvent oxyder le soufre élémentaire mais la bactérie b<sub>2</sub> ne s'est jamais montrée capable de s'attaquer directement au soufre dans les conditions de mes recherches.

Toutes ces propriétés ressortent des résultats expérimentaux résumés dans le Tableau 7.

TABLEAU 7.—Transformation des hyposulfites et du soufre en sulfates en présence de différents aliments azotés. Le soufre des sulfates formés est exprimé en milligrammes de SO<sub>3</sub> pour une culture de 50 cc. Toutes les cultures ont été faites en présence de CaCO<sub>3</sub> et ont duré 90 jours

Aliment azoté	Peptone		Succinate d'ammonium		Chlorhydrate d'ammonium	
	Soufre précipité	Hyposulfite de sodium	Soufre précipité	Hyposulfite de sodium	Soufre précipité	Hyposul de sodium
b <sub>2</sub>	0	8	0	135	0	40
T <sub>2</sub>	1	90	37	15	0	23
OS <sub>3</sub>	8	132	43	125	32	151

### C. LA FORMATION DES SULFATES AUX DÉPENS DU SOUFRE PAR VOIE D'ASSOCIATION MICROBIENNE

Nous avons vu que, dans les conditions expérimentales où je me suis placé, un nombre relativement important de bactéries ammonisatrices et un actinomycète sont capable de solubiliser le soufre à l'état d'hyposulfites.

D'autre part, nous savons que la bactérie  $b_2$  ne peut s'attaquer au soufre par ses propres moyens mais qu'elle peut transformer en sulfates les hyposulfites solubles.

Si, dans une solution minérale II additionnée d'un aliment azoté convenable (peptone ou succinate d'ammonium) et de soufre, on ensemence simultanément un microorganisme producteur d'hyposulfites et la bactérie  $b_2$ , on constate que le soufre se solubilise à l'état de sulfates dans le liquide de culture alors qu'aucune des deux espèces microbiennes mises en oeuvre ne peut effectuer à elle seule une telle transformation (9, 11). Dans certains cas à côté des sulfates solubles il reste encore des proportions variables d'hyposulfites non attaqués par  $b_2$ . (Tableau 8).

TABLEAU 8.—Transformation du soufre en sulfates par voie d'association microbienne. Les résultats sont exprimés en milligrammes de  $S_2O_2$  ou de  $SO_3$  formés aux dépens du soufre dans une culture de 50 cc. de solution minérale additionnée de 0.5% de peptone et 5% de soufre

Microorganismes étudiés	En cultures pures		En association avec $b_2^*$	
	$S_2O_2$	$SO_3$	$S_2O_2$	$SO_3$
Bacillus subtilis	7.2	0	0	0
Bacillus mesentericus	65.0	0	0	120.0
Bacillus megaterium	214.0	0	43.0	171.0
Bacillus mycoides	29.0	0	33.0	20.0
Bacterium coli	17.0	0	0	48.0
Proteus	17.0	0	0	51.0
Actinomyces griseus	65.0	0	0	151.0
M M	175.0	0	0	271.0
$b_2$	0	0	0	0

\* La bactérie  $b_2$  est incapable de s'attaquer directement au soufre.

Lorsque, dans des expériences analogues aux précédentes, on substitue à la bactérie  $b_2$  les bactéries  $T_2$  ou  $OS_3$ , on obtient toujours des transformations du soufre en sulfates beaucoup plus rapides dans les cultures où ces bactéries travaillent en association avec un producteur d'hyposulfites que dans celles où elles agissent seules. Mais en présence des bactéries  $T_2$  et  $OS_3$  qui peuvent elles mêmes transformer un peu de soufre en sulfates, le rôle de l'association, bien qu'encore très net reste cependant moins saisissant que dans les cas où l'étude a été faite en présence de  $b_2$ .

Je dois enfin ajouter pour terminer que, dans celles des cultures en association que j'ai le plus complètement étudiées, je n'ai observé la formation d'aucun produit intermédiaire entre les hyposulfites et les sulfates. Mais cela ne veut pas dire qu'avec d'autres microorganismes que ceux que j'ai alors employés (MM, Actinomyces griseus et  $b_2$ ) ou dans des conditions de culture différentes de celles que j'ai réalisées, la formation de ces produits intermédiaires ne pourra être mise en évidence.

## CONCLUSIONS DE CETTE ÉTUDE

L'ensemble des recherches résumées dans cette communication nous apporte en définitive deux notions qui se complètent mutuellement:

(1) Dans un sol riche en azote organique et bien aéré, l'évolution du soufre élémentaire peut relever d'un processus microbien complexe qui fait apparaître, avant les sulfates, des composés du soufre solubles et oxydables. Parmi les produits intermédiaires ainsi formés on ne peut pas déceler la présence des sulfures mais on trouve au contraire régulièrement des hyposulfites.

(2) Dans un milieu artificiel riche en azote organique et dépourvu de nitrates, la transformation du soufre en sulfates peut être obtenue rapidement par l'action combinée de deux espèces microbiennes dont l'une solubilise le soufre à l'état d'hyposulfites (espèce ammonisatrice) tandis que l'autre transforme les hyposulfites formés en sulfates (espèce plus ou moins strictement autotrophe).

En présence d'une matière organique azotée la transformation du soufre en sulfates peut donc résulter d'un enchevêtrement d'actions microbiennes dont certaines sont incontestablement attribuables à des espèces hétérotrophes; le phénomène diffère alors profondément de celui qu'on peut observer dans un milieu purement minéral. Entre le cycle évolutif de l'azote et le cycle évolutif du soufre élémentaire dans le sol il existe donc des relations étroites et il n'est pas difficile de prévoir que, si le premier influence le second, le second devra de même avoir sur le premier une répercussion plus ou moins profonde.

## LITTÉRATURE CITÉE

- (1) Beijerinck. 1904. Centbl. Bakt. [Etc.] 2: 593.
- (2) ———. 1904. Ibid. 2: 597.
- (3) Boullanger et Dugardin. 1912. Compt. Rend. Acad. Sci. [Paris] 155: 327.
- (4) Demolon. 1913. Ibid. 156: 725.
- (5) ———. 1921. Ibid. 173: 1408.
- (6) Deniges. 1890. Bul. Soc. Pharmacie Bordeaux. p. 313.
- (7) Gehring. 1915. Centbl. Bakt. [Etc.] 2: 402.
- (8) Guittonneau, G. 1925. Compt. Rend. Acad. Sci. [Paris] 180: 261.
- (9) ———. 1925. Ibid. 181: 261.
- (10) ———. 1926. Ibid. 182: 661.
- (11) ———. 1927. Ibid. 184: 45.
- (12) Jacobsen. 1912. Folia Microb. 1: 487, 8: 155.
- (13) Lieske. 1912. Ber. Deut. Bot. Gesell. 30: 12.
- (14) Lipman, J. G. Soil Sci. 2: 205.
- (15) ———, Waksman, S. A., Joffe, J. S. 1921. Ibid. 12: 475.
- (16) Nathanson. 1903. Centbl. Bakt. 2: 109.
- (17) Rippel, A. 1924. Ibid. 2: 290.
- (18) Trantwein. 1921. Ibid. 2: 513.
- (19) Vogel. 1914. Ibid. 2: 60.
- (20) Waksman, S. A., and Joffe, J. S. 1921. Proc. Soc. Exp. Biol. Med. 18: 1.
- (21) ———, ———. 1921. Science 53: 216.
- (22) ———, ———. 1922. Jour. Bact. 7: 239.
- (23) ———, ———, and Starkey R. L. 1923. Jour. Agri. Research [U. S.] 24: 297.

# DER GEGENWÄRTIGE STAND UNSERER KENNTNISSE VON DEN EISENBAKTERIEN

R. LIESKE

*Biologische Reichsanstalt für Land- und Forstwirtschaft, Berlin-Dahlem*

Eisen- und Manganverbindungen spielen eine bedeutende Rolle im Erdboden, von kohlensäurehaltigem Wasser werden sie leicht in Form von Oxydulverbindungen aufgenommen. Seit langem ist bekannt, dass in solchen Wässern bestimmte Bakterienarten vorkommen, die wegen ihrer spezifischen Beziehungen zu diesem Element als "Eisenbakterien" bezeichnet werden.

Das gemeinste, überall in eisenhaltigen Wässern auftretende und über die ganze Erde verbreitete Eisenbakterium ist *Leptothrix ochracea* Kützing (Syn. *Chlamydothrix ochracea* Migula). Dieser Organismus bildet etwa 1  $\mu$  breite und 2–5  $\mu$  lange Stäbchen, die kettenförmig aneinandergereiht und von einer dünnen Gallerthülle umgeben sind. Die einzelnen Stäbchen können aus den Scheiden austreten und bilden begeißelte Schwärmer, die frei im Wasser umherschwimmen. Diese setzen sich später fest und können wieder zu neuen Ketten auswachsen. Die Scheide ist in natürlichen Gewässern immer stark eisenoxydhaltig. Eine von Molisch als *Leptothrix* (*Chlamydothrix*) *sideropous* beschriebene Form scheidet die eisenspeichernde Gallertschicht nur an der Basis der fest-sitzenden Stäbchenkette ab. Eine von Cholodny (1) in neuester Zeit als *Leptothrix crassa* beschriebene Form dürfte mit *Leptothrix ochracea* identisch sein.

Weitere biologisch sehr interessante und weit verbreitete Eisenorganismen sind *Gallionella ferruginea* Ehrenberg und *Spirophyllum ferrugineum* Ellis. *Spirophyllum* bildet lange, gewundene, bandartige Fäden, die nach den Untersuchungen von Cholodny (2) von einem stäbchenförmigen, am Ende des gewundenen Fadens sitzenden Organismus abgechieden werden. Die gewundenen Bänder sind also nur die Stiele, auf denen die lebenden Zellen aufsitzen. *Gallionella ferruginea* bildet Fäden mit meist kreisrundem Querschnitt, diese biegen sich in der Mitte um und die beiden freien Enden winden sich zopfartig umeinander. Bei oberflächlicher Betrachtung sieht ein solcher *Gallionella*-Fäden der *Spirophyllum* sehr ähnlich, dass diese beiden Organismen identisch sind, wie manche Forscher annehmen, ist aber keineswegs bewiesen, besonders betont sei, dass an typischen *Gallionella*-Fäden lebende Zellen wie bei den *Spirophyllum*-Fäden bisher noch nicht beobachtet wurden.

Eine dritte wichtige Gruppe von Eisenbakterien stellen *Crenothrix polyspora* und *Clonothrix fusca* dar. Es handelt sich hierbei um grössere Organismen, die morphologisch den Algen nahe stehen. Zellen von 2–5  $\mu$  Breite und 2–10  $\mu$  Länge sind wie bei *Leptothrix* kettenförmig aneinandergereiht und von einer gemeinsamen Scheide umgeben. Die Scheide speichert wie bei *Leptothrix* Eisen bzw. Mangan. *Clonothrix fusca* Schorler unterscheidet sich von *Crenothrix polyspora* Cohn hauptsächlich dadurch, dass diese Form falsche Verzweigungen bildet, während die *Crenothrix*-Fäden immer unverzweigt sind. *Crenothrix* speichert unter natürlichen Verhältnissen vorwiegend Eisen, *Clonothrix* vorwiegend Mangan, beide Formen können aber auch die beiden Elemente gleichzeitig enthalten.

Sehr verbreitet in der Natur, namentlich an Wasserpflanzen haftend, aber praktisch weniger bedeutungsvoll sind kleine, kokkenförmige Bakterien, die von einem eisenspeichernden Gallert umgeben sind und die Molisch (6) als *Siderocapsa Treubii* und *Siderocapsa major* bezeichnet. Eine Anzahl weiterer in der Literatur als Eisenbakterien bezeichneter Organismen sind entweder nicht genau bestimmt oder praktisch bedeutungslos, so dass sie hier nicht erwähnt zu werden brauchen.

*Physiologie:* Die auffällige Eigenschaft aller Eisenbakterien, grosse Mengen Eisen bzw. Mangan aus Lösungen niederzuschlagen, ist praktisch von grösster Bedeutung und hat naturgemäss seit langem das Interesse der Physiologen erregt. Die ersten exakten Untersuchungen über Eisenbakterien wurden von Winogradsky (7, 8) ausgeführt. Er beobachtete einzelne lebende Fäden von *Leptothrix ochracea* in einem Flüssigkeitstropfen unter dem Mikroskop und stellte fest, dass sie nur wuchsen, wenn sie in eisenoxydulhaltigem Wasser lagen. War den Fäden keine Gelegenheit zur Eisenspeicherung gegeben, so stellten sie ihr Wachstum ein. Winogradsky schloss aus seinen Versuchen, dass die Eisenspeicherung nur in eisenoxydulhaltigem Wasser durch Oxydation des Eisens innerhalb der Scheide zustande kommt. Der Oxydationsvorgang ist an die lebende Zelle gebunden und findet hauptsächlich im Protoplasma statt. Ohne Eisenoxydul wachsen die Eisenbakterien nicht. Die Lebensprozesse der Eisenbakterien werden hauptsächlich oder ausschliesslich auf Kosten der bei der Oxydation von Eisenoxydul zu Eisenoxyd freiwerdenden Wärme (aktuelle Energie) im Gange gehalten.

Im direkten Gegensatz zu diesen Angaben Winogradskys stehen die Ausführungen von Molisch (6). Er arbeitete als erster mit Reinkulturen von *Leptothrix ochracea*, und es gelang ihm, diesen Organismus in Peptonwasser ohne Zusatz von Eisen- oder Mangansalzen zu kultivieren. Molisch stellte ferner fest, dass man solche Kulturen viele Generationen hindurch fortsetzen konnte, ohne die Lebensfähigkeit der *Leptothrix* irgendwie zu beeinträchtigen. Aus seinen Versuchen schliesst er, dass

die Speicherung von Eisen bzw. Mangan für die Eisenbakterien ernährungsphysiologisch bedeutungslos ist.

Zur Entscheidung der gegensätzlichen Ansichten von Winogradsky und Molisch wurden vom Verfasser (4) weitere Versuche mit Reinkulturen von *Leptothrix ochracea* angestellt. Es zeigte sich zunächst, dass die Angaben Molischs über seine Kulturergebnisse vollkommen zutreffend sind. Es ergab sich aber weiter, dass die Speicherung der Eisen- bzw. Manganverbindungen keineswegs ohne Bedeutung ist, wie Molisch annahm. Schlechte organische Nährlösungen, die an sich kein Wachstum von *Leptothrix* ergaben, z. B. sehr dünne Extrakte aus alten Blättern, ermöglichten ein gutes Wachstum, sobald man ihnen Eisen- oder Manganoxydulsalze zusetzte. Diese Berunde stimmen mit dem Vorkommen von *Leptothrix* in der Natur überein. Wir finden eine üppige *Leptothrix*-Vegetation nur in eisenhaltigen Wässern, in sonst gleich zusammengesetzten eisenfreien Wasser wird eine Massenvegetation dieses Organismus niemals beobachtet. Dass *Leptothrix ochracea* in eisenfreien Nährlösungen wachsen kann, ist keineswegs ein Beweis dafür, dass die Eisenspeicherung unter natürlichen Verhältnissen bedeutungslos ist.

Unentschieden ist bisher noch die Frage, ob *Leptothrix* rein kohlenstoff-autotroph leben kann, dass dieser Organismus also befähigt wäre, mit Hilfe der Oxydationsenergie freie Kohlensäure zu assimilieren, wie Winogradsky annahm. Sowohl die Untersuchungen Winogradskys als auch verschiedene andere Beobachtungen lassen dies als möglich erscheinen, ein wirklich exakter Beweis hierfür ist aber bisher noch nicht geliefert worden. Von *Spirophyllum ferrugineum*, das sich in der Natur meist nur in Eisenwässern entwickelt, die sehr arm an organischer Substanz sind, konnte gezeigt werden, dass es sich in Kulturen ohne Zusatz von organischer Substanz entwickeln kann (5).

Interessante theoretische Betrachtungen über die Physiologie der Eisenbakterien veröffentlichte in neuester Zeit Francis (3). Er nimmt an, dass die Eisenverbindungen mit den primitivsten Lebensvorgängen in enger Beziehung stehen und führt unter anderem folgendes aus: "Es kann angenommen werden, dass einige der chemischen Prozesse, wie Oxydation und Hydratisation, welchen Eisen und einige seiner Verbindungen unterworfen sind, wenn sie mit Wasser und Kohlensäure in Berührung kommen, in der Vergangenheit und auch noch in der Gegenwart die primitivsten kolloidalen Zustände des Protoplasmas gebildet haben, des Protoplasmas, welches in seiner lebendigen Ausbildung als Kolloid-Komplex aus verschiedenen Formen von Kolloiden, wie Suspensoide, Emulsoide, Sols und Gels besteht. Die chemischen Grundlagen dieser beiden Prozesse, der Oxydation und der Hydratisation, welche die primären Faktoren bei der Erzeugung des kolloidalen Zustandes aus dem Eisen sind, stellen die grundlegenden Faktoren für die Erhaltung des Lebens dar." Dass typische Eisenbakterien wie *Spirophyllum*, *Gal-*

*Gallionella* und *Leptothrix* tatsächlich primitivste Formen des Lebens darstellen ist nicht unmöglich.

*Praktische Bedeutung der Eisenbakterien.* Von praktischer Bedeutung sind vor allem *Spirophyllum ferrugineum*, *Gallionella ferruginea* und *Leptothrix ochracea* dadurch, dass sie aus eisenhaltigen Wässern das gelöste Eisenoxydulkarbonat in ihren Scheiden als Oxydhydrat ausfällen. Es sind auf diesem Wege in der Natur riesige Ablagerungen von Eisenhydroxyd (Raseneisenstein) entstanden. In Wasserleitungen und offenen Gewässern bilden diese Eisenbakterien zuweilen sehr lästige Verunreinigungen, die durch Filteranlagen beseitigt werden müssen. Man vermeidet die Entwicklung der Bakterien durch Enteisung des Wassers auf chemischem Wege, was einfach durch Lüftung (Rieselung) des Wassers geschehen kann. Das im Wasser gelöste Eisenoxydulkarbonat fällt zum grössten Teile bei Berührung mit dem Luftsauerstoff aus und kann dann leicht abfiltriert werden.

*Crenothrix polyspora* und *Clonothrix fusca* sind typische Wasserleitungsorganismen, Massenv egetationen derselben werden häufig in Wasserleitungen, im Gegensatz zu den vorerwähnten Eisenbakterien aber fast niemals in der freien Natur beobachtet. *Crenothrix* kann durch ihr massenhaftes Auftreten die Leitungen verstopfen, desgleichen *Clonothrix*, welche noch viel unangenehmer werden kann dadurch, dass sie mit Vorliebe grosse Mengen von Mangan speichert. Enthält der Erdboden Manganverbindungen, so werden diese wie das Eisen als Oxydulverbindungen durch die Kohlensäure gelöst. Solches manganoxydulhaltiges Wasser wäre an sich ganz vollwertig, es ist vollkommen klar und das Mangan fällt auch in Berührung mit der Luft nicht aus. *Crenothrix* und besonders *Clonothrix* oxydieren nun aber das Mangan und speichern die schwarzen Oxydverbindungen in ihren Scheiden. Hierdurch kann das Wasser vollkommen schwarz gefärbt werden, selbst dicke Leitungsröhre werden verstopft. Als Trinkwasser und für technische Zwecke kann solches Wasser gänzlich unbrauchbar werden. Eine solche *Clonothrix*-Epidemie ist vor einiger Zeit in der Stadt Dresden aufgetreten, das Wasser floss in einzelnen Stadtteilen zuweilen ganz dick und schwarz aus den Leitungshähnen, in der Nähe gelegene Papierfabriken gerieten durch das geschwärzte Wasser in grosse Betriebsschwierigkeiten. Die Entmanganung des Wassers ist technisch weit schwieriger als die Enteisung, da das Mangan durch den Luftsauerstoff nicht ausfällt. Man hat nun mit sehr gutem Erfolge versucht, dem Wasser das Mangan auf biologischem Wege zu entziehen. Man verwendet hierzu dieselben Bakterien, welche die erwähnten Unannehmlichkeiten verursachen. Auf grossen, geschlossenen Sandfiltern wird eine Vegetation von *Clonothrix* und *Crenothrix* angesiedelt. Das Wasser wird, wenn es aus den Brunnen kommt, über diese Filter geleitet, wo ihm durch die Bakterien das Mangan entzogen

wird. In dem manganfreien Wasser kann sich dann in den Leitungen keine weitere Bakterienvegetation entwickeln.

Vorstehende kurze Ausführungen mögen dazu beitragen, zum weiteren Studium der noch in vielen Beziehungen unvollkommen erforschten Eisenbakterien in weitesten Kreisen anzuregen. Es handelt sich jedenfalls um eine Organismengruppe von besonderem wissenschaftlichen Interesse und von grosser praktischer Bedeutung.

## ZITIERTE LITERATUR

- |                  |       |  |
|------------------|-------|--|
| (1) Cholodny.    | 1926. | Die Eisenbakterien. Jena.  |
| (2) ———.         | 1924. | Zur Morphologie der Eisenbakterien <i>Gallionella</i> und <i>Spirophyllum</i> . Ber. Deut. Bot. Gesell., Bd., 42, S. 35. |
| (3) Francis.     | 1926. | Ein Beitrag zur Theorie der Beziehung des Eisens zur Entstehung des Lebens. Botanisches Archiv, Bd. 15, S. 377.          |
| (4) Lieske.      | 1919. | Zur Ernährungsphysiologie der Eisenbakterien. Centbl. Bakt. etc. II Abt., Bd. 49, S. 431.                                |
| (5) ———.         | 1911. | Beiträge zur Kenntnis der Physiologie von <i>Spirophyllum ferrugineum</i> . Jahrb. Wiss. Bot. Bd. 49, S. 91.             |
| (6) Molisch.     | 1910. | Die Eisenbakterien. Jena.  |
| (7) Winogradsky. | 1888. | Über Eisenbakterien. Botanische Zeitung, Bd. 46, S. 261.   |
| (8) ———.         | 1922. | Eisenbakterien als Anorgoxydanten. Centbl. Bakt., etc. II Abt., Bd. 57, S. 1.  |



# THE RÔLE OF MICROORGANISMS IN TRANSFORMATIONS OF IRON IN NATURE

R. L. STARKEY<sup>1</sup> AND H. O. HALVORSON  
*University of Minnesota, U. S. A.*

## INTRODUCTION

Iron is one of the most abundant elements in the earth's crust, comprising some 4.5 per cent of the lithosphere. Next to aluminum this is the most abundant metal, only oxygen and silicon occurring in greater abundance. As a factor in soil fertility, iron is important both directly as an essential element and indirectly in its numerous influences on physical and chemical characteristics of soils. Although occurring in such abundance in native rocks and soils and although it is required by plants in small amounts it frequently exists in such unavailable form as to be a limiting factor to plant development. In this connection soil reaction appears to be of major importance. In calcareous soils very small or negligible amounts of iron appear in true solution or at least as ions.

The existence of very slightly ionized dissolved compounds is not unlikely and may be of considerable importance in the movement of iron in soils. However, the availability of such unionized forms of iron for plant nutrition is not well defined. Transformations of iron in nature are also of considerable importance in the formation of large deposits of iron ore of aquatic origin; closely associated with such deposits must have been activities responsible for the initial solution of iron from soils and rocks and removal by bodies of water. Deposition of iron with rusting of pipes and the fouling of drinking waters may become matters of considerable economic importance. Precipitation of iron and other substances may be responsible for the formation of hardpans. Soil color may be largely determined by the chemical and physical state of the iron.

In general, it appears that in well aerated arable soils this iron is in the ferric form and generally occurs in the reduced state only where free oxygen is largely excluded, under such conditions as exist in bogs and other poorly aerated soils. What the conditions may be in the zone of root absorption is not clear but certainly it would appear that the oxygen pressure would be much lower than in the regions more distant from the roots.

The following discussion indicates that ferrous iron might not only occur in the absence of appreciable amounts of free oxygen but also would

<sup>1</sup> Now of the New Jersey Agr. Exp. Station, U. S. A.

be in greater abundance in the presence of greater concentrations of hydrogen ions. Although studies of the soil or soil solution as a whole may suggest that iron generally exists in the insoluble oxidized form, the environmental conditions may be quite different in the limited root zone of absorption. This intimate relationship between root and soil solution is largely obscured by ordinary analytical treatment of soil. It seems likely that there would be a higher concentration of iron in solution in this zone of absorption resulting from both a lower oxygen concentration as a result of microbial activity and root excretion and, further, a somewhat greater hydrogen ion concentration. The formation of certain organic compounds of iron in this region may also greatly modify the mechanism of absorption.

Precipitation of iron under natural conditions may result from any of a large number of changes. Not only may there be a variety of products formed, but the agencies primarily responsible for the reactions may be quite different. Among the reactions which have occupied the interests of microbiologists there stand out prominently the changes which have been ascribed to a group of organisms called iron bacteria, first studied by Winogradsky and ably discussed in the preceding report by Lieske. Their activity in soils is hypothetical and even their existence as a physiologic entity questioned. Even assuming their development, this group of organisms is only one of many agencies which may effect a precipitation of compounds of iron. Strictly chemical changes of inorganic compounds may occur in nature independent of microorganisms. These transformations of inorganic compounds may take place indirectly as a result of modifications of the environment by the development of microorganisms. Decomposition of organic compounds may be followed by a similar deposition. In fact, since the reactions of oxidation and reduction are reversible, slight modifications of environmental conditions may not only inhibit precipitation but even favor solution. Although iron bacteria, as generally considered, are all responsible for precipitation of iron, only a small group of the organisms active in precipitation of iron may be considered to be iron bacteria.

### THEORETICAL CONSIDERATION OF THE PRECIPITATION OF IRON

It appears to be possible to elaborate somewhat on the relationships which microorganisms may have with precipitation and solution as well as oxidation and reduction of iron in the light of the following equations which have been developed by the junior author. In the presence of solid ferric hydroxide:

$$\frac{[A_{Fe^{++}}]}{[A_{Fe^{+++}}]} \cdot [A_{H^{+}}] = \frac{K}{[A_{O_2}]^{\frac{1}{2}}} \quad (1)$$

$$\frac{[A_{Fe++}]}{[A_{H+}]^2} = \frac{K'}{[A_{O_2}]^{\frac{1}{4}}} \quad (2)$$

Under atmospheric conditions creating constant oxygen pressure these may be expressed as follows:

$$\frac{[A_{Fe++}]}{[A_{Fe+++}]} \cdot [A_{H+}] = K'' \quad (3)$$

$$\frac{[A_{Fe++}]}{[A_{H+}]^2} = K''' \quad (4)$$

It can be observed from these equations that the ratio of the activities of ferrous and ferric iron, which in dilute solutions will not be appreciably different from the ratio of the concentrations of the inorganic ferrous and ferric iron in solution, will depend upon the reaction and oxygen pressure, two factors which may be altered to a considerable degree by development of microorganisms. Since the reactions of oxidation of iron are reversible, transformations of oxidation may change to reduction depending upon the environmental conditions. In general it may be stated that lowering the oxygen pressure, that is, creating more anaerobic conditions, will: (1) tend to increase ferrous iron at the expense of the ferric, and (2) tend to decrease the hydrogen ion concentration. Further, increasing the oxygen pressure, such as creating aerobic conditions will lead toward: (1) a greater amount of ferric iron per unit of ferrous and (2) more acid reaction. Increasing the hydrogen ion concentration with constant oxygen pressure will also tend to diminish the ratio of ferrous iron in solution to dissolved ferric iron by dissolving more of the precipitate and at the same time, at equilibrium, holding much more ferrous iron in solution. Lowering the hydrogen ion concentration with constant oxygen pressure would bring about an oxidation of ferrous iron with resulting precipitation of the ferric form, also increasing the ratio of the concentrations of ferrous to ferric ions.

Modifications of hydrogen ion concentration or oxygen pressure as a result of microbial growth actually bring about changes in oxidation, reduction, solution and precipitation of iron such as are predicted from a study of the equations.

### CONDITIONS EFFECTING SOLUTION OF IRON

Let us first consider those factors associated with solution of iron. Under aerobic conditions iron may go into solution as a result of increasing the hydrogen ion concentration by the formation of either organic or inorganic acids by microorganisms.

Under anaerobic conditions experimental results have shown that considerable amounts of iron may dissolve in the presence of decomposing

organic material either carbohydrate or proteinaceous in nature. This is principally the result of lowering the oxygen concentration. Much larger amounts dissolve where carbohydrates are decomposing under anaerobic conditions since here considerable amounts of organic acids are produced. As iron enters solution under anaerobic conditions from the insoluble ferric hydrate, it is largely reduced to the ferrous form and the ratio of concentrations of ferrous to ferric ions is greatly increased. It is evident from the preceding equations that this proportionally lowers the hydrogen ion concentration. Even though considerable amounts of organic acids may be produced, the buffering effect of the iron compounds conceals the actual change in acid production. While under ordinary atmospheric conditions negligible amounts of ferric ions exist close to neutrality ( $6.1 \times 10^{-8}$  p.p.m.) as much as 740 p.p.m. entered solution at pH 6.0 under anaerobic conditions.

### CONDITIONS EFFECTING PRECIPITATION OF IRON

Factors concerned with precipitation of iron though probably of no greater importance than solvent agencies have attracted more general attention in previous studies. It may be observed from the equations cited earlier that iron in solutions at equilibrium will become oxidized by increasing the oxygen concentration or lowering the hydrogen ion concentration. Consequently, solutions carrying iron under reduced oxygen tension such as anaerobic cultures or subterraneous streams will show oxidation of iron upon exposure to normal atmospheric conditions. Under these conditions greater amounts of ferric ions are produced than are sufficient to saturate the solution. There is then a precipitation of ferric hydrate. This reaction is consequently associated with an increase in the concentration of hydrogen ions and the reaction proceeds until a new equilibrium of hydrogen, hydroxyl, ferrous and ferric ions is established which is stable under the atmospheric conditions. Such changes proceed either in sterile conditions or in the presence of microorganisms.

Precipitation of ferric hydrate may also occur under aerobic conditions in the presence of decomposing nitrogenous organic materials. In such cases the deposition appears to be very closely associated with the increase in hydroxyl ion concentration resulting from the formation of ammonia by microorganisms in the decomposition process. In the presence of decomposing peptone, even though the microorganisms tend to lower the oxygen concentration by their development and the precipitation tends to increase the acidity, such large amounts of ammonia are produced that quite complete precipitation occurs where growth is vigorous.

Of no less interest is the precipitation of ferric hydrate under aerobic conditions from compounds of such a nature as ferric ammonium citrate. Such compounds which are stable at reactions close to neutrality under atmospheric conditions, owe their stability to their slight ionization.

Precipitation of iron from such compounds subsequent to microbial activity is the result of the decomposition of the organic radical which liberates an abundance of ions of iron into a system which will not hold them in solution; there naturally follows an hydrolysis and precipitation. It does not appear striking that organisms of a great variety having the capacity of precipitating iron from organic compounds may be found widely distributed in nature; it would seem more unusual if they were not numerous.

Precipitation appears to be more commonly associated with increases in oxygen concentration but may also occur in quite extreme anaerobic environments. Such compounds as iron sulfides may be precipitated under anaerobic conditions since the low solubility product of such compounds results in their deposition under conditions which would even permit solution of ferric hydrate.

In solutions which are saturated with respect to iron, precipitation will occur subsequent to oxidation unless extremely few ions of iron exist. Oxidation of iron may or may not result in precipitation depending upon the extent of the ionization of the oxidized form. It may frequently happen, as found in these studies, that, subsequent to exposure of anaerobic cultures to aerobic conditions, practically complete oxidation of large amounts of ferrous iron occurred with no precipitation. It is obvious that compounds must have been formed which were of extremely low ionization. It is most probable that these compounds were organic.

It can be stated further that precipitation may occur independently of oxidation of iron. This is the case of precipitation as the result of decomposition of organic compounds of ferric iron by heterotrophic microorganisms. Whether or not the iron in solution occurs in organic unionized or inorganic ionized forms, iron will tend to become oxidized by increasing the oxygen pressure or lowering the hydrogen ion concentration.

It is apparent that little mention has been made of the so-called autotrophic iron bacteria. That such forms exist which develop from the energy of oxidation of ferrous to ferric iron is not contested by us and much evidence has been gathered which appears to explain their metabolism. However, as regards the agencies primarily responsible for oxidation and precipitation of iron under natural conditions it appears to be assuming too much to state that iron bacteria are of major importance in the deposition of iron merely because they are generally found in regions of iron precipitation. Supposing that iron bacteria are even active in the formation of natural deposits we may assume that they can develop where chemical precipitation would not proceed or assume that they accelerate the change which might occur spontaneously. This latter assumption appears more logical. As far as is known, all iron bacteria are aerobic. In fact, the reaction by which they exist is one of oxidation; one of the characteristics always associated with their activity is the

accumulation of precipitated ferric hydrate about the cells. Also the reaction by which they exist is reversible. Let us assume a condition of equilibrium as regards ferrous and ferric iron in solution. If iron bacteria should become active here the concentration of both ferrous iron and oxygen would become reduced and ferric hydrate would be precipitated. The solution would consequently be in a state conducive to reduction and the precipitated iron would again go into solution. Unless the solution is not in equilibrium but in a state conducive to oxidation, accumulation of a precipitate of ferric hydrate cannot occur. It would appear, then, that iron bacteria exist upon a very narrow margin utilizing energy from a reaction which is occurring spontaneously at no negligible speed.

### SUMMARY

From the preceding considerations it would appear that precipitation of iron under natural conditions may be much more commonly a result of actions independent of iron bacteria than is generally accepted. Further, so many agencies are active in the process that biologically the mere precipitation of iron appears to be of little significance unless the reactions responsible for the change are understood.

These observations find application to transformations of iron in soil particularly as the factors of hydrogen ion and oxygen concentrations appear to be of major importance in the changes. Further, both of these factors may be greatly altered by microbial activity.

# LA RÉDUCTION PAR VOIE BIOLOGIQUE DES PHOSPHATES MINÉRAUX DANS LE SOL

K. T. ROUDAKOW

*Bacteriologo-Agronomical Station, Moscou, U. S. S. R.*

En étudiant les transformations biologiques de l'acide phosphorique, nous observons plus d'une fois la diminution rapide et accentuée de la quantité de l'acide phosphorique dissoute dans le substrat nutritif. Ce phénomène s'observait seulement dans le cas, quand le substrat nutritif était versé en une couche assez haute, c.-à.-d. quand l'activité vitale des microorganismes s'écoula dans des conditions anaérobies. Puisque la diminution de la quantité de  $P_2O_5$  était trop grande et dépassa considérablement la quantité, qu'a pu être assimilé par les microorganismes développés dans le substrat et passé de cette manière dans l'état d'absorption biologique, nous avons ému la supposition de la possibilité de la réduction de l'acide phosphorique en combinaisons moins oxydées.

Une série d'expériences préliminaires menées dans de conditions sévèrement anaérobies, on permi d'établir définitivement, qu'a la part de l'absorption biologique on ne peut attribuer plus de 7.5 pour cent de la quantité de l'acide phosphorique dissoute dans l'eau, qui disparaît comme résultat de l'activité vitale d'organismes du sol.

La partie la plus grande de l'acide phosphorique en cas d'absence dans le substrat nutritif d'une grande quantité de combinaisons oxydées disparaît comme résultat d'autres procédés biologiques. En étudiant de plus près ces procédés, nous avons établi que sous l'influence d'agents biologiques, une partie considérable de l'acide phosphorique peut être réduite en  $H_2PO_3$  et  $H_2PO_2$  et même jusqu'a l'hydrogène phosphorique. En qualité d'illustration citons quelques chiffres (Tableaux 1 et 2).

TABLEAU 1.—Réduction de l'acide phosphorique à  $H_2PO_3$  et  $H_2PO_2$

Le sol appliqué pour infecter le substrat nutritif	$P_2O_5$ réduit à $H_2PO_3$ et $H_2PO_2$	Remarque
Le sol de la Station Bacteriolo-Agronomique	mg. pour litre 447	Substrat nutritif contenait 1041 mg. $P_2O_5$ pour litre
Tchernosem (terre noir) de Chatiloff Control	423	

TABLEAU 2.—Réduction de l'acide phosphorique à  $\text{PH}_3$ 

Le sol appliqué pour infecter le substrat nutritif	$\text{P}_2\text{O}_5$ réduit à $\text{PH}_3$	Remarque
Le sol de la Station Bacteriolo-Agronomique	mg. pour litre 12	Substrat nutritif contenait 1020 mg $\text{P}_2\text{O}_5$ pour litre
Tchernosem (terre noir) de Tchelabinsk Control	32 de traces	

Ensuite nous avons dégagé 30 races de bactéries, qui réduisent  $\text{P}_2\text{O}_5$  à l'état de  $\text{H}_3\text{PO}_3$  et  $\text{H}_3\text{PO}_2$  et même jusqu'à l'hydrogène phosphorique. Toute les cultures dégagées se montraient identiques morphologiquement et physiologiquement, mais différaient beaucoup en activité.

Comme la plus énergique se montre la cultur No. 13 se caractérisant par de particularités suivantes: Des bacilles courts ( $1,1-1,5\mu$  en longueur et  $0,4-0,6\mu$  en épaisseur), mobiles, ne formants pas de spores, se développant bien sur l'agar phosphaté-mannité (eau aquedue 1 litre, mannite 20,0;  $\text{NH}_4\text{H}_2\text{PO}_4$  1,5). Ils végètent aussi bien sur des milieux nutritifs ordinaires (agar viande-pepton, gélatine, bouillon, pomme de terre). On fait l'inoculation en éduant seulement ou en piquant (dans la cas dernier il résulte un déchirement du milieu). Ils ne changent pas le lait (en 10 fois 24 heures); ne liqúeficent pas la gélatine; en rapport de l'oxygène se montrent des anaérobies facultatives; tout de même après plusieurs inoculations dans des conditions aérobies leur énergie reductrice tombe. Outre sur la mannite, ces microbes se développent sur la glucose, saccharose, lactose mais alors sa force réductrice pour les phosphates est bien plus faible. Ils ne se développent pas sur les sels de chaux des acides organiques (lactique, tartrique, citrique) et sur la glycérine. La température minimal du développement est  $15^\circ$ , optimale  $35^\circ$  et maximale vers  $40^\circ$ .

La balance du phosphore dans le substrat nutritif liquide, comme résultat de l'activité vitale du microbe No. 13 se reproduit dans le Tableau 3.

TABLEAU 3.—Balance du phosphore dans le substrat nutritif, comme résultat de l'activité vitale du microbe No. 13; mg.  $\text{P}_2\text{O}_5$  pour litre

Réduit jusqu'à $\text{PH}_3$	Réduit jusqu'à $\text{H}_3\text{PO}_3$ et $\text{H}_3\text{PO}_2$	Réduit comme $\text{P}_2\text{O}_5$	Absorbé biologique et passé dans de combinaisons indéfini
102	467	1306 2500	625 (calculé)



L'étude ultérieure du microbe que nous avons dégagé nous a permis d'établir que le procédé de réduction de l'acide phosphorique peut par son caractère être rapproché au procédé de dénitrification et désulphatisation. N'ayant pas à sa disposition de l'oxygène libre ou de combinaisons ( $\text{NO}_3$ ,  $\text{SO}_4$ ) que se desoxydent plus aisément que  $\text{P}_2\text{O}_5$ , le microbe puise l'oxygène que lui est nécessaire de phosphates minéraux en oxydant en même temps les combinaisons organiques non azotées.

Dans les conditions anaérobies ou en présence d'autres (autre  $\text{P}_2\text{O}_5$ ) combinaisons minérales oxydées, la réduction de phosphates se passe bien plus faiblement, parce que le microbe préfère dans ce cas utiliser l'oxygène des sources plus accessibles.

Pour mieux prouver cette affirmation, nous donnons les résultats d'une de nos expériences mise pour éclaircir l'influence de l'addition de  $\text{KNO}_3$  et de  $\text{MgSO}_4$  sur la réduction de la culture pure du microbe No. 13. Le schéma de l'expérience est que voici :

- (1) Substrat nutritif
- (2) Do +  $\text{KNO}_3$
- (3) Do +  $\text{MgSO}_4$

Le substrat nutritif est versé dans des tubes infectés par la culture du microbe No. 13 et mis au thermostat à  $35^\circ$ . La durée est de 5 fois 24 heures. Le résultat de l'analyse est donné dans le Tableau 4.

TABLEAU 4.—Influence de l'addition de  $\text{KNO}_3$  et  $\text{MgSO}_4$  sur la réduction de  $\text{P}_2\text{O}_5$

No. des tubes	Le contenu en $\text{P}_2\text{O}_5$ dans la culture de 5 en mg. pour litre		Réduit jusqu'à $\text{H}_3\text{PO}_3$ et $\text{H}_3\text{PO}_2$ en mg. pour litre
	Infectés	Stériles	
1	1389,0	1529,0	
2	1536,4	1706,5	118,1
3	1453,3	1584,7	33,3

En même temps que nous avons déterminé  $\text{P}_2\text{O}_5$  nous faisons le compte des microbes dans le hématimètre de Thomas. Le résultat du calcul est dans le Tableau 5.

TABLEAU 5.—Influence de l'addition de  $\text{KNO}_3$  et  $\text{MgSO}_4$  sur le nombre des microbes

No. des tubes	Quantité de bactéries dans 1 cc.
1	$120 \times 10^6$
2	$260 \times 10^6$
3	$216 \times 10^6$

Comme on voit selon le tableau, dans les tubes avec  $\text{KNO}_3$  et  $\text{MgSO}_4$  malgré le développement plus fort de bactéries, la réduction de l'acide

phosphorique s'écoule considérablement plus faible que dans les tubes à substrat nutritif normal.

Dans la dernière série d'expérience nous tâchions d'établir le phénomène de réduction biologique de l'acide phosphorique dans le sol même en l'infectant par la cultur pure des microbes No. 13. Les résultats sont resumés dans Tableau 6.

TABLEAU 6.—Réduction de  $P_2O_5$  dans sols infectés par la cultur pure du microbe No. 13

	Extrait aqueux				En tout $P_2O_5$ pour kg. de sol absol. sec		Réduit en $PH_3$ mg. $P_2O_5$ p. kg. de sol absol. sec	
	Solution aqueux $P_2O_5$ , mg. p. 1 kg. de sol absol. sec		$H_2PO_4$ et $H_2PO_3$ mg. p. kg. de sol. absol. sec					
	Stériles	Infectés	Stériles	Infectés				
Sol depart. Moscou	138. 75	129. 75		7. 30	2429	2386		43
Tchernosem de Chatiloff	135. 10	91. 80		62. 75	2797			72

Outre la définition indirecte de l'hydrogène phosphorique formé dans le sol comme résultat de l'activité vital du microbe No. 13, nous l'avons, au cours d'une expérience, déterminé immédiatement en le surprenant par l'eau bromé. Une expérience posé avec le sol de la station expérimental du départ de Moscou nous a permis de surprendre vers 9 mg. d'hydrogène phosphorique émanés sous l'influence du travail du microbe No. 13.

Quant aux conditions indispensables à l'activité réductrice des microbes dans le sol, nous avons maintenant établi que les procédés réducteurs se renforcent parallèlement à l'humidité c.-à-d. parallèlement à l'aération. Ensuite l'énergie de la réduction de l'acide phosphorique augmente dans les conditions de cultur de notre microbe simultanément avec un autre aérobe. Enfin nous avons posé une série d'expériences dans le but d'éclairer la possibilité de la réduction de l'acide phosphorique dans les sols naturels, que n'ont subi aucune intervention, c.-à-d. stérilisation, infection consecutive, etc.

Le résultat de nos expériences a confirmé notre supposition.

Les sols naturels misent dans les conditions anaérobies perdaient dans un certain temps quelque quantité d'acide phosphorique ( $P_2O_5$ ) à cause du passage de cette dernière dans des combinaisons moins oxydées. En résumé nous communiquerons le résultat de l'étude comparative sur l'énergie réductrice (relativement à  $P_2O_5$ ) de sols différents. Nous avons établi que les sols différents sont loin de posséder une énergie réductrice égal et même on aperçoit leur soumission à certains lois.

Ainsi les sols vierges (de Bakourian, de Tchakva, du sommet de Karagatch, de Goudaious, le Tchernosem de Tchelabinsk) se montrent en possession d'une énergie réductrice peu considérable. Les sols cultivés, au contraire (le Tchernosem de Chatiloff, le sol du champs d'expérience de Petrowsky Académie Agronomique) se montrent comme réducteurs très énergiques.

# PURIFICATION OF CHEMICALS FOR USE IN THE PREPARATION OF NUTRIENT SOLUTIONS

## I. THE CRYSTALLIZATION OF PHOSPHORIC ACID

N. A. CLARK

*Iowa State College, U. S. A.*

### INTRODUCTION

The importance of purity in chemicals which are used in nutrient media is becoming more appreciated. Frequently the need of this purity is brought home to the chemist in a way he cannot mistake. For example, to the worker with plants and soil in the last few years the effect of very small quantities of substances like iron, boron (2), manganese (6), or organic matter (3, 4) in the life history of plants is beginning to be emphasized, and the need for care in the selection of chemicals is evident. Methods, therefore, which aim at increasing the ease with which substances can be freed from impurities, are of general utility.

Usually purification is comparatively simple, and this is particularly true for substances which will crystallize from solution. Where crystallization is impossible, the preparation of the compound must often be traced through the literature and methods developed from the results of experiments found there. More often, however, a fairly simple operation will result in a much improved product, and this is especially true of the C. P. chemicals of commerce. Sometimes it is desirable to treat "analyzed chemicals" in the same way when a very pure sample is required.

In the case of a large number of soluble substances the amounts going into solution at different temperatures can be looked up in a table of solubilities, and crystals obtained from a supersaturated solution. These crystals may be dried on filter paper or on a porous plate, although the use of a centrifuge is quicker and often more effective. For the last method, for very small quantities of material, a hard glass, large sized test tube can be constricted about half way down, and a perforated porcelain plate inserted. During centrifuging the liquid collects in the bottom of the tube and the crystals are caught on the plate.

In order to handle rather larger amounts of material, a transparent bakelite container was employed and found to be very satisfactory. A container of this kind was made to order in Chicago, to fit the large holder of a centrifuge. This was run at about 2000 r. p. m. and separated the

precipitate from the mother liquor very quickly. The dimensions are given in Fig. 1. The sieve removes easily for cleaning if the container is allowed to dry after use. Water should not be left in contact with the

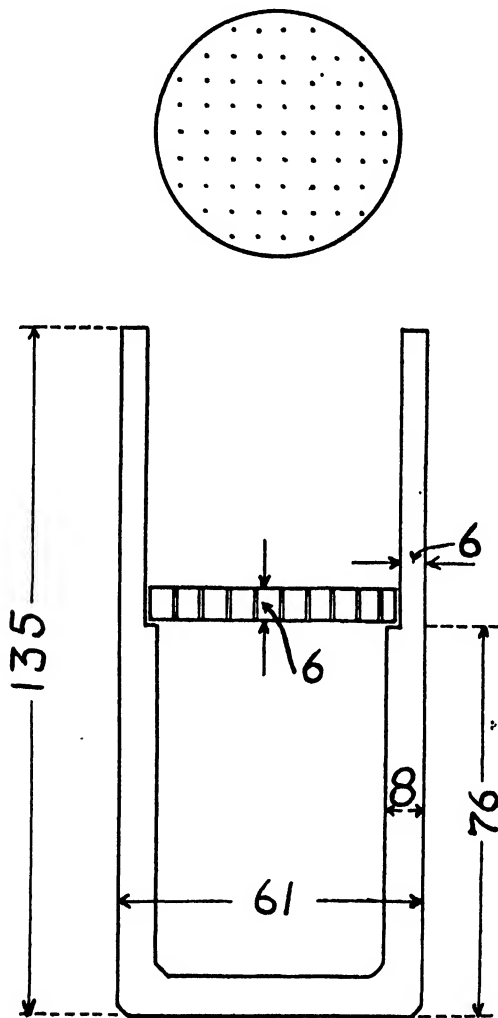


FIGURE 1.—Bakelite container for centrifuge. Dimensions in millimeters. Holes for removable strainer are 0.5 mm. diameter—5 mm. centers

bakelite continuously, as a slight swelling will result and the sieve then tends to stick.

### ORTHOPHOSPHORIC ACID

Until recently, the preparation of pure phosphoric acid has been a somewhat tedious process. Gmelin-Kraut's Handbuch (5) gives directions for its production from the element and from several phosphorus

compounds. Abbott and Bray (1) in a study of the phosphates in 1909, prepared the acid by oxidizing yellow phosphorus with 16 times its own weight of nitric acid of specific gravity 1.20. A much more convenient process, and one which will give as pure a product, is the crystallization of a supersaturated solution of the acid. This method was suggested and

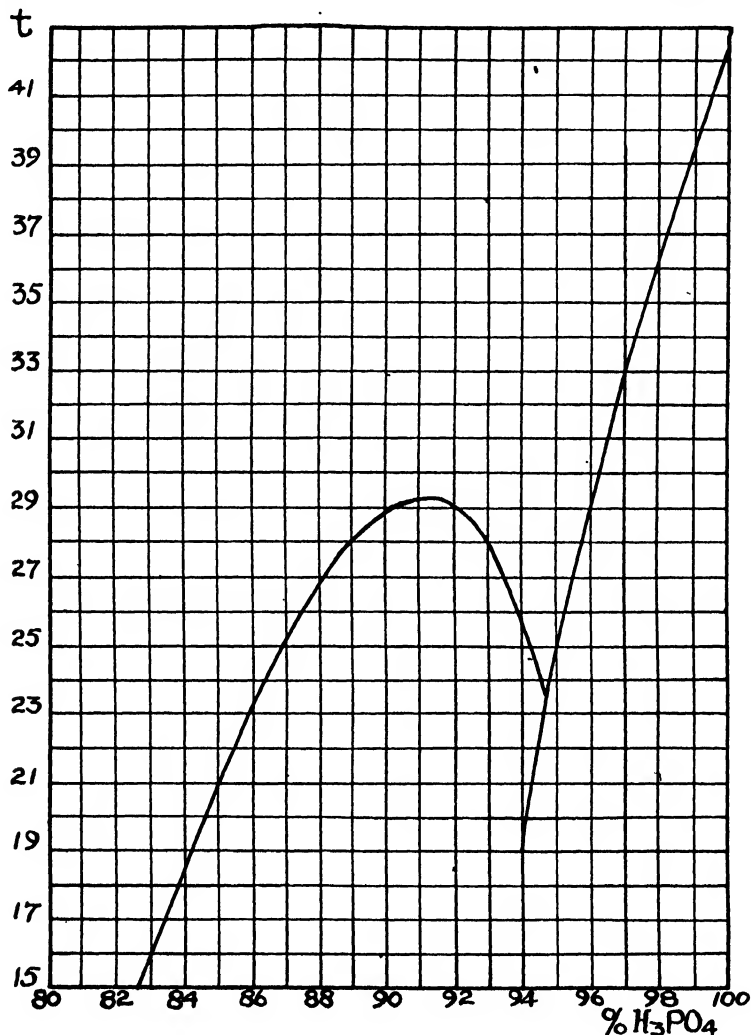


FIGURE 2.—Solubility curves of hydrated and anhydrous orthophosphoric acid. From Ross, Jones and Durgin

investigated in detail by Ross, Jones and Durgin (7) and has proved to be quick and easy to carry out. It can be applied to large quantities of the acid, or on a small laboratory scale to purify the C. P. grade. In the latter case a few alterations in the original procedure seem to be desirable.

Orthophosphoric acid will deposit crystals of both the hydrated acid, m. p.  $29^{\circ}\text{C}.$ , and the anhydrous  $\text{H}_3\text{PO}_4$ , m. p.  $42^{\circ}$ . The hydrated crystal forms hard lumps, is sticky, and is difficult to get pure, whereas the anhydrous is much more easily handled. The solubility curves of the two crystals (Fig. 2) were obtained by Ross, Jones and Durgin, who recommend for crystallization of the anhydrous acid, a solution of specific gravity 1.85 at  $20/20^{\circ}$ , cooled below  $40^{\circ}$  and seeded with a crystal of the anhydrous acid. They state, however, that the temperature to which the phosphoric acid solution is subjected before crystallization has a marked effect on the rate at which crystals form, and that if the solution is heated for some time above  $130^{\circ}$  no crystallization will take place. They recommend, therefore, that the concentration of the acid to specific gravity 1.85 should be done below  $100^{\circ}$ .

The process of concentrating the solution in an open vessel below  $100^{\circ}$  is a slow one. Placing the vessel in a vacuum oven at  $90^{\circ}$ , and drawing dry air through the oven, while maintaining the vacuum with a water pump, increases the speed but little. A third method was tried in order to remove the water more quickly; the acid was dropped over glass beads, through a tower which was kept surrounded by boiling alcohol, the vapor of which was returned by a condenser to the flask below. This process was quicker but still unsatisfactory, and search was made for conditions under which crystallization would take place from a solution evaporated above  $100^{\circ}$  in an open vessel.

Evaporation at just below boiling point, up to  $170^{\circ}$ , in an open vessel is fairly rapid. When a solution of specific gravity 1.85, concentrated in this way, was cooled to  $25^{\circ}$  and then seeded with an anhydrous crystal, it crystallized readily. According to the solubility curves given by Ross, Jones and Durgin (Fig. 2), below 23.5 a solution of specific gravity 1.85 is also supersaturated with the hydrate; thus, when the temperature was dropped suddenly to near zero by the use of ice, the hydrate started to precipitate without preliminary seeding. Both types of crystals have been observed forming at the same time in different parts of the same dish; a solution with a concentration over 91.6 per cent, when allowed to crystallize below  $23^{\circ}$ , will always give a mixture of the two crystals, the ratio depending upon the concentration of the acid—the greater the per cent acid, the smaller the quantity of the hydrate formed.

For the purification of the acid it is not necessary to exclude all traces of the hydrate, so that a very rapid method of crystallization can be adopted. The temperature of a solution of specific gravity 1.85 is lowered to  $15^{\circ}$  or less and seeded with an anhydrous crystal. The deposit is the anhydrous  $\text{H}_3\text{PO}_4$ , along with some of the hydrate. The formation is stopped before solidification and the crystals quickly centrifuged. These crystals are melted above  $43^{\circ}$ , a small quantity of water added to bring the solution to the 1.85 specific gravity at  $20^{\circ}$ , and recrystallized as often

as required. The last crystallization, if done above 25°, will be pure anhydrous  $\text{H}_2\text{PO}_4$ . The crystals can be kept over  $\text{P}_2\text{O}_5$ ; both the hydrate and the anhydrous if exposed to the air take up sufficient water to form solutions.

Ross, Jones and Durgin draw attention to the fact that there is frequently suspended matter in the commercial acid which cannot be filtered out unless the acid is dilute: they suggest that the solution should be allowed to stand for some time at a temperature between 50° and 90° in order to let the impurities settle, or diluting to 1.75 specific gravity and filtering through sand. A more satisfactory method is to use glass wool for removing the suspended matter; dilution is not needed as solutions will pass readily through the glass wool when suction from a water pump is applied. Filtration is rarely needed except before the first crystallization.

### SUMMARY

A bakelite container has been described which is suitable for the removal of mother liquor from crystals by the use of the centrifuge.

Some alterations have been suggested in the method of Ross, Jones, and Durgin for the purification by crystallization of orthophosphoric acid.

### LITERATURE CITED

- (1) Abbott, G. A., and Bray, W. C. 1909. The ionization relations of ortho- and pyrophosphoric acids and their sodium salts. *J. Am. Chem. Soc.* 31: 729.
- (2) Brenchley, W. E. 1914. On the action of certain compounds of zinc, arsenic and boron on the growth of plants. *Ann. Bot.* 28: 283.
- (3) Clark, N. A. 1926. Plant growth-promoting substances, hydrogen ion concentration and the reproduction of Lemna. *Plant Physiol.* 1: 273.
- (4) ———, and Roller, E. M. 1924. Auximones and the growth of the green plant. *Soil Sci.*, 17: 193.
- (5) Gmelin-Kraut. 1910. *Handbuch der Anorganischen Chemie.* 1: 3.
- (6) McHargue, J. S. 1926. Manganese and plant growth. *Indus. Engin. Chem.*, 18: 172.
- (7) Ross, W. H., Jones, R. M. and Durgin, C. B. 1925. The purification of phosphoric acid by crystallization. *Indus. Engin. Chem.* 17: 1081.

# UNTERSUCHUNGEN ÜBER DIE BEDEUTUNG DER BODENATMUNG FÜR DIE KOHLENSÄUREER- NÄHRUNG DER KULTURPFLANZEN

O. LEMMERMANN

*Landwirtschaftliche Hochschule, Berlin-Dahlem, Deutschland*

In neuerer Zeit haben bekanntlich einige Forscher die Meinung vertreten, dass die aus dem Boden austretende  $\text{CO}_2$  von den Pflanzen *unmittelbar* und in hohem Masse für die Assimilation ausgenutzt werden könne. Ich bin auf Grund meiner Untersuchungen anderer Meinung.

Nach meiner Ansicht hat die aus dem Boden austretende  $\text{CO}_2$  unmittelbar für die Assimilation der Pflanzen keine besondere praktische Bedeutung. Präziser gesagt, bezieht sich diese Ansicht zunächst auf diejenige  $\text{CO}_2$ -Menge, um die die normale Bodenatmung durch eine Düngung mit Stalldünger bezw. Gründünger gesteigert werden kann.

Diese meine Ansicht stützt sich namentlich auf folgende von uns experimentell festgestellte Tatsachen.

## 1. UNTERSUCHUNGEN ÜBER DIE MENGE DER DURCH STALLDÜNGER IM BODEN PRODUZIERTEN $\text{CO}_2$ NEBST VEGETATIONSVERSUCHEN ÜBER IHRE VERWERTUNG DURCH KULTURPFLANZEN

Bei Versuchen über die Bedeutung des Stalldüngers bezw. Gründüngers als  $\text{CO}_2$ -Quelle, die wir sowohl auf freiem Felde als auch in Vegetationsgefäßen angestellt haben, fanden wir stets,

(a) dass zwar die  $\text{CO}_2$ -Produktion des Bodens durch die Düngung mit Stalldünger bezw. Gründünger erheblich vermehrt wurde.

Wir fanden z.B., dass auf unserem Versuchsfelde in Dahlem im Mittel von je 54 Bestimmungen an  $\text{CO}_2$  produziert wurden:

auf dem mit Mineraldünger gedüngten Felde	182 mg. $\text{CO}_2$
Do Stalldünger	Do
	245 mg. $\text{CO}_2$

innerhalb einer Stunde auf 1 qm Fläche.

Das bedeutet eine Erhöhung der  $\text{CO}_2$ -Produktion durch die Düngung mit Stalldünger um 35%.

(b) Wir konnten aber weiterhin durch Vegetationsversuche feststellen, dass diese Mehrproduktion an  $\text{CO}_2$  ohne Einfluss war auf die Ernteerträge. Um das festzustellen, ist es nötig, den Faktor  $\text{CO}_2$  aus den verschiedenen



Faktoren, aus denen sich die Wirkung des Stalldüngers zusammensetzt, zu isolieren. Wie das zu machen ist, habe ich wiederholt beschrieben.

Bei den Versuchen, die wir im Jahre 1925 angestellt haben, verfahren wir z.B. in folgender Weise.

Wir haben an je zwei Stellen der eben genannten Felder, die entweder nur mit Mineraldünger bzw. daneben noch mit Stalldünger gedüngt waren, je 6 Vegetationsgefässe (im ganzen also 24) aufgestellt, die mit ein und demselben Boden gefüllt und mit ein und derselben Mineraldüngung in der üblichen Weise gedüngt waren. Sie trugen, wie das sie umgebende Feld, ebenfalls Gerste. Sie waren in kleinen, *ganz schmalen* Gräben innerhalb der Felder so aufgestellt, dass die Oberfläche der Gefässe sich in gleicher Höhe wie der Acker befand. Auch die Pflanzen der Gefässe befanden sich demnach in gleicher Höhe mit den Feldpflanzen, von denen sie so *vollkommen umgeben* waren, dass der Standort der Gefässe schon bei geringer Entfernung gar nicht mehr zu erkennen war. Die Gräben waren nur so gross, dass die Gefässe eben in ihnen Platz fanden. Um die Kohlensäureernährung der Gefässpflanzen auf dem Stalldüngerfelde möglichst günstig zu gestalten, wurde die Sohle des ausgehobenen Grabens unterhalb der Gefässe noch extra mit einer Schicht Stalldünger versehen. *Infolge dieser Versuchsanordnung war also die Bodenernährung (bzw. Wurzelernährung) der Pflanzen gleich, während die Lufternährung (bzw. Blätterernährung) verschieden war.*

Bei diesen Versuchen fanden wir folgendes. Es wurden geerntet im Mittel von je 12 Gefässen:

Standort der Gefässe	{	Mineraldüngerfeld	49,30 g. $\pm$ 1,23
		Stalldüngerfeld	45,53 g. $\pm$ 1,63

Die Ernten waren also so gut wie gleich. Eine Wirkung der auf dem Stalldüngerfelde mehr produzierten  $\text{CO}_2$  auf die Ernten war nicht eingetreten.

Dieser Befund steht in Übereinstimmung mit den Ergebnissen früherer Versuche, die in ähnlicher Weise angestellt worden waren.

## 2. WIRKUNG EINES ERSATZES DES STALLDÜNGERS DURCH MINERALDÜNGER AUF DIE ERNTEN

Wir haben weiter bei Feldversuchen gefunden, dass man einen Teil des Stalldüngers, unbeschadet des Ertrages, durch Mineraldünger ersetzen kann, trotzdem die Bodenatmung auf den Stalldüngerfeldern grösser ist. Das wäre nicht möglich, wenn die Boden- $\text{CO}_2$  von grösserer Bedeutung für die Assimilation wäre. Dieselbe Beobachtung machten auch Remy, Gerlach, Mach.

### 3. UNTERSUCHUNGEN ÜBER DEN CO<sub>2</sub>-GEHALT DER LUFT ÜBER VERSCHIEDEN GEDÜNGTEN FELDERN WÄHREND DER TAGESSTUNDEN

Wir haben ferner unter den Verhältnissen der Praxis mehrere Jahre hindurch die durchschnittliche Zusammensetzung der Luft gemessen einmal über solchen Feldern, die mit Stalldünger gedüngt waren, zweitens über solchen, die nur Mineraldünger erhalten hatten; wir taten das in der Weise, dass wir monatelang während der Tagesstunden täglich 4–5 Stunden die Luft (1–20 cm. über dem Boden) durch Pettenkofer'sche Röhren leiteten. Wir haben dabei gefunden, dass der "Tagesdurchschnitt" des CO<sub>2</sub>-Gehaltes der Luft über dem Stalldüngelfeld nicht grösser war als über dem Mineraldüngelfeld, trotzdem die CO<sub>2</sub>-Produktion des Bodens auf dem Stalldüngelfeld 35% grösser war als auf dem Mineraldüngelfeld. Wir fanden z.B., dass in 100.000 Teilen Luft vorhanden waren im Tagesmittel:

TABELLE 1.—CO<sub>2</sub> in 100.000 Teilen der Luft Während der Tageszeit

	über dem Mineraldüngelfeld	über dem Stalldüngelfeld	Mittel von
1921	35	35	36 Beobachtungen
1922	32	33	33 Do
1925	33	33,6	92 bzw. 78 Do

Der durchschnittliche CO<sub>2</sub>-Gehalt der Luft war also während der Tageszeit (anders liegen die Verhältnisse während der Nacht) über dem Mineraldüngelfeld und dem Stalldüngelfeld annähernd gleich gross.

Aus diesen und anderen experimentellen Untersuchungen muss man m.E. schliessen, dass der Stalldünger sowie der Gründünger als CO<sub>2</sub>-Quelle für die Assimilation nicht die Bedeutung hat, wie die Vertreter der sogenannten CO<sub>2</sub>-Düngung das annehmen.

Wenn nun *einerseits* durch Versuche erwiesen ist, dass aus Stalldünger bzw. Gründünger grössere Mengen CO<sub>2</sub> im Boden entstehen und aus dem Boden austreten, und wenn *andererseits* sich ergeben hat, dass die Pflanzen diese CO<sub>2</sub> nicht in nennenswertem Masse verwerten können, so muss man annehmen, dass die CO<sub>2</sub>-Menge, die aus dem Boden austritt, sehr schnell durch die Luftbewegung verdünnt und verweht wird. Das ist auch ganz verständlich, denn die Luftbewegung ist im Verhältnis zu der in der Zeiteinheit produzierten CO<sub>2</sub>-Menge zumeist recht gross, selbst innerhalb eines Pflanzenbestandes. Die Luftbewegung wird auch nicht nur durch den Wind, sondern auch durch Luftwirbel, infolge Erwärmung, hervorgerufen. Sie verläuft auch keineswegs nur horizontal, sondern stets auch vertikal, wie das unsere in dieser Hinsicht angestellten Versuche beweisen. Wir fanden z.B., dass die aufwärts gerichtete Bewegungsköm-

ponente im Roggen 1–4 cm. in der Sekunde betrug. Eine solche Bewegung genügt, um die aus dem Boden austretende  $\text{CO}_2$  (die bei unseren Versuchen in einer Sekunde 0,026 bis 0,034 cc. je qm. betrug) bis zur Unmerklichkeit zu verdünnen.

#### 4. DER $\text{CO}_2$ -GEHALT DER LUFT WÄHREND DER NACHTSTUNDEN IM BESTANDE VERSCHIEDENER PFLANZEN

Wir haben dann weiter untersucht, wie sich der  $\text{CO}_2$ -Gehalt der Luft während der Nachtzeit im Pflanzenbestande ändert, also zu einer Zeit, wo die Pflanzen nicht assimilieren. Die Luft wurde während der Nachtstunden 6–7 Stunden durch Pettenkofersche Röhren geleitet. Wir fanden im Mittel zahlreicher Versuche folgende Zahlen als "Durchschnittswerte für die Nacht":

TABELLE 2.— $\text{CO}_2$ -Gehalt der Luft während der Nacht

In 100.000 Teilen Luft waren vorhanden:	über dem Bestande 2 m. Höhe	im Bestande halbe Höhe	In der Nähe des Bodens	Probenahme in cm. über dem Boden	
		a	b	a	b
<i>Roggenfeld</i> in 100.000 T. Luft mehr in %	31,2	31,7  0,5 1,6%	32,4  1,2 4,0%	70 cm.	25 cm.
<i>Kartoffelfeld</i> in 100.000 T. Luft mehr in %	31,6	34,1  2,5 8%	35,0  3,4 11%	25 cm.	5 cm.
<i>Luzernefeld</i> in 100.000 T. Luft mehr in %	32,0	33,8  33,8? 5,6%	35,5  35,5? 11%	25 cm.	5 cm.

Die Zahlen zeigen, dass während der Nacht in allen Fällen eine messbare Zunahme des  $\text{CO}_2$ -Gehaltes der Luft im Bestande nachweisbar war im Gegensatz zu den am Tage ausgeführten Versuchen. Das ist ja auch durchaus verständlich, da die Pflanzen während der Nacht nicht assimilieren, und die von ihnen ausgeatmete  $\text{CO}_2$  zur Anreicherung der Luft an  $\text{CO}_2$  beiträgt.

#### 5. BEDEUTUNG FÜR DIE ERNTEN

Man kann aus diesen Zahlen auch ein ungefähres Bild darüber gewinnen, welche Bedeutung die Bodenatmung für die Assimilationsfähigkeit

im günstigsten Falle besitzen kann. Man kann bekanntlich auf Grund der Versuche von Brown-Escombe, Lundegardh, Mitscherlich annehmen, dass der *Erntezuwachs höchstens proportional der CO<sub>2</sub>-Konzentration steigt*. Das heisst, eine Vermehrung der CO<sub>2</sub>-Konzentration um 1% kann höchstens eine Erntevermehrung von 1% zur Folge haben usw. Das würde also in dem vorliegenden Falle bedeuten, dass die *gesamte Bodenatmung etwa folgende Mehrerträge bewirken könnte*:

für Roggen	1,6 bis 4%
für Kartoffeln	8 bis 11%
für Luzerne	5,6 bis 11%

Praktisch liegt die Sache aber etwas anders. Praktisch läuft die Frage darauf hinaus.

- (a) um wieviel kann die "natürliche" bezw. die an und für sich vorhandene Bodenatmung durch eine Düngung mit Stalldünger bezw. Gründünger gesteigert werden,
- und
- (b) welche Bedeutung hat dieser *Zuwachs* an CO<sub>2</sub> für die Ernte?

Auch für die Beantwortung dieser Fragen geben unsere Untersuchungen Anhaltspunkte. Wir fanden z.B., dass bei *unserem Luzerne-Versuch* (während der Assimilationszeit) *die gesamte Bodenatmung täglich 200 kg. CO<sub>2</sub> je ha.* war. Das bedeutet eine Erhöhung der CO<sub>2</sub>-Konzentration um 5,6% und ferner, dass somit auch nur eine Erhöhung des Ertrages um höchstens 5,6% theoretisch möglich war.

Wir fanden nun weiter, dass *eine Düngung mit 300 dz. Stalldünger je ha.* im günstigsten Falle eine *Vermehrung der Bodenatmung von 50 kg. CO<sub>2</sub> täglich* zur Folge hatte (in Wirklichkeit wohl meistens weniger). D.h., die CO<sub>2</sub>-Konzentration und damit die Erntesteigerung kann durch die Stalldüngergabe im *günstigsten Falle nur 1/4* von derjenigen Erntesteigerung betragen, die durch die Bodenatmung an sich bewirkt werden kann. *Mit anderen Worten: durch die Stalldünger-CO<sub>2</sub> kann theoretisch die Ernte um etwa 1% erhöht werden.*

Das stimmt vollkommen mit unseren vorher erwähnten Vegetationsversuchen, bei denen wir niemals eine Wirkung der Stalldünger-CO<sub>2</sub> auf den Ertrag feststellen konnten.

## 6. BIS ZU WELCHEM GRADE KANN DIE PFLANZE DER LUFT DIE CO<sub>2</sub> ENTZIEHEN?

Einen Einwand gegen diese Darlegungen kann man nur erheben, wenn man sich auf den Boden der sogenannten CO<sub>2</sub>-Rest-Theorie von Reinau stellt. Denn nach dieser allerdings unbewiesenen Hypothese soll ein CO<sub>2</sub>-Gehalt der Luft von 0,026 Vol. % auch bei bester Beleuchtung nicht mehr ausreichen, den Pflanzen CO<sub>2</sub> zuzuführen, weil im Blattinnern nur

derjenige  $\text{CO}_2$ -Anteil assimiliert wird, der oberhalb des "Schwellenwertes" 0,026% liegt. *Nach dieser Hypothese wurde die Assimilationsbedingung schon um das Doppelte verbessert, wenn der Luftgehalt von 0,030% auf 0,034% steigt.*

Diese Hypothese ist aber nicht richtig. Schon den Versuchen von Th. de Saussure u.a. kann man entnehmen, dass die Pflanzen der Luft die  $\text{CO}_2$  bis auf Spuren entziehen kann.

Wir fanden das Gleiche bei unseren Versuchen. Wir überstülpten in vollem Wachstum sich befindliche Senfpflanzen und Haferpflanzen mehrere (7) Stunden hindurch mit Glaskolben und stellten den  $\text{CO}_2$ -Gehalt der Luft innerhalb und ausserhalb des Glaskolbens fest.

Wir fanden, dass der  $\text{CO}_2$ -Gehalt der Luft *innerhalb des Kolbens* betrug

beim Senf-Versuch      0,001%

Do Hafer-Versuch      0,001 bis 0,006%

Der  $\text{CO}_2$ -Gehalt der *Aussenluft* war während des Versuches 0,031%.

## 7. ANTEIL DER WURZELTÄTIGKEIT AN DER BODENATMUNG

Dann möchte ich noch auf folgenden Punkt hinweisen. Wenn man von der Bodenatmung spricht, dann denkt man fast immer nur an die  $\text{CO}_2$ , die durch die Zersetzung der Humusstoffe im Boden entsteht. Die Anschauung ist aber nur für den nackten Boden richtig. Wenn man aber die Bodenatmung eines mit Pflanzen bestandenen Bodens untersucht, dann kommt noch eine andere  $\text{CO}_2$ -Quelle hinzu: nämlich die Atmung der Wurzeln.

Und die ist oft recht bedeutend.

Wir fanden, dass sie

bei Roggen, je nach Alter      1/4—1/2

Do Luzerne      bis zu      4/5

der gesamten Bodenatmung ausmachen kann

*Zusammenfassend* kann ich also sagen, dass die aus dem Boden austretende  $\text{CO}_2$  des Stalldüngers und der Gründüngung, trotzdem ihre Menge nicht gering ist, für die  $\text{CO}_2$ -Ernährung der Kulturpflanzen keine nennenswerte Bedeutung besitzt.

Wenn ich somit auf Grund meiner langjährigen Erfahrungen auf dem Standpunkt stehe, dass die von dem Boden ausgeatmete  $\text{CO}_2$  für die Assimilation durch die Blätter nicht von besonderer Bedeutung ist, so möchte ich andererseits betonen, dass ich durchaus der Meinung bin, dass der Kohlenstoffgehalt eines Bodens von höchster Bedeutung für seinen Fruchtbarkeitszustand ist, und dass wir dem Kohlenstoffhaushalt des Bodens dieselbe Bedeutung schenken müssen wie seinem Stickstoff-,

Phosphorsäure-, Kali- und Kalkhaushalt. Es ist auch deshalb notwendig, auf den Kohlenstoffhaushalt eines Bodens besonders acht zu geben, weil die Zersetzung durch die Zufuhr der künstlichen Düngemitte (wie ich das ebenfalls bereits in den Jahren 1919 durch Versuche festgestellt habe), insbesondere von N, wesentlich erhöht wird.

## MICROBIOLOGICAL ASPECTS OF GREEN MANURING

J. G. LIPMAN AND A. W. BLAIR

*New Jersey Agricultural Experiment Station, Rutgers University, U. S. A.*

The effects claimed for green manuring are many-fold. The plant cover representing green manure crops undoubtedly helps to conserve the constituents of the soil solution which might otherwise be removed in the drainage water. It is also obvious that a green manure often serves to protect the surface soil against erosion. A leguminous green manure crop should increase the store of soil nitrogen. It should, likewise, increase in a more or less substantial way the content of organic matter in the soil and should exert a beneficial effect on the soil texture. When a green manure crop is plowed under, it undergoes a process of fermentation which, in its turn, reacts in a more or less far-reaching way on the transformation of substances in the soil. The supply of available nitrogen compounds to the crop, the composition of the soil air, the reaction of the soil, the development of substances more or less toxic in character, the supply of the mineral constituents of plant food, and, finally, the nature of the soil microflora are all affected by the fermentation processes in green manure residues mixed with the soil. It is well known, furthermore, that the effectiveness of green manures may be increased by properly adjusting the green manure crop to the soil, the season and the climate. Other factors which have a bearing on the effectiveness of the utilization of different green manures are soil reaction, the supply of available plant food derived from the soil itself or from animal manures and chemical fertilizers, the time of plowing under the green manure, the extent of the inoculation of leguminous green manures and, finally, the breeding and selection of crops used for green manure purposes.

In the light of the general considerations just noted, the data collected at the New Jersey Agricultural Experiment Station and reported in the following pages will perhaps be more clearly understood. In the fall of 1907 an area of land that had not been under cultivation for many years, and which was in a low state of fertility, was laid out in twentieth-acre plots. The soil of these plots is a gravelly loam representing residual material from triassic red shale worked over by stream action. The area included in these experiments is level in character—though it was not entirely uniform when the experiments were initiated. In the spring of 1908 a crop of Indian corn was planted on the area in question, consisting

of eight one-twentieth-acre plots. Annual applications of 300 lb. of superphosphate and of 100 lb. of muriate of potash have been made since the beginning of these experiments.<sup>1</sup> During the period 1908 to 1910, inclusive, there was supplied, in addition, fish meal at the rate of 200 lb. per acre. In the following five years no nitrogenous fertilizer was applied. From 1916 on the land has received applications, in addition to the superphosphate and muriate of potash as already noted, of nitrate of soda at the rate of 160 lb. per acre. The fish meal contained about 10 per cent of nitrogen; the nitrate of soda, 15.5 per cent of nitrogen. During the period 1908 to 1926 there were, therefore, applied in all about 230 lb. of nitrogen per acre, or 11.5 lb. per plot, that is, not much more than one-half pound of nitrogen per plot per annum.

Indian corn has been grown on these plots continuously since 1908. Four of the eight plots have been receiving additions of nitrogen and of organic matter through the growing of leguminous catch crops in the corn. The other four plots were receiving additions of at least organic matter through the growing of rye as a catch crop in the corn. Aside from the treatments already mentioned, small quantities of farmyard manure were employed with the thought that the inoculating effect of these small quantities of manure might make itself felt in the decomposition of the green manure after it was plowed under. The amounts of manure used on both the legume and non-legume sections were 1000, 2000 and 4000 lb. per acre respectively. The following table contains information concerning the yields of grain and of nitrogen from each treatment calculated on the acre basis. In 1910 it was necessary to grow oats rather than corn on these plots. Hence, the crop of that year is excluded from the average. The leguminous green manure employed has usually been vetch and a mixture of several clovers, although alfalfa has at times been included in the mixture. In one or two seasons a slight admixture of soybeans was also employed. In the non-legume section rye has been used regularly. The stand of legumes was good in some of the seasons. In most of the seasons, however, the shading of the ground by the Indian corn and the limited supply of moisture of the gravelly soil interfered with the development of a satisfactory stand of the leguminous catch crop.

An examination of the yields of grain and nitrogen as recorded in the table will show that the yields in the legume section were substantially larger than those in the non-legume section. The average for 18 years shows a yield of 37.9 bu. of grain per acre in the legume plot which had received no manure treatments and 26 bu. per acre in the corresponding non-legume plot. Similar differences are found in the plots which have been receiving the small applications of 1000, 2000 and 4000 lb. of manure per acre. In the 18 years the plots with 1000 lb. of manure received a total of 90 lb. of nitrogen; the plot with the 2000 lb. of manure received 180

<sup>1</sup> The application of potash was omitted in 1918.



lb. of nitrogen, and that with 4000 lb., 360 lb. of nitrogen. Other experiments carried on at the New Jersey Station show that, under field conditions, scarcely more than one-third of the manure nitrogen applied is recovered in the crop. Hence, the amounts actually transformed into crop substances on the plots in question may be assumed to have been 30, 60 and 120 lb. per acre respectively.

On the legume section the no-manure plot shows an average annual nitrogen content in the crop of 49.1 lb. per acre. The corresponding quantity on the plot with 1000 lb. of manure shows a return of 55.2 lb. of nitrogen per acre. The 2000 and 4000 lb. applications of manure did not increase the nitrogen return to any marked extent over that obtained from the plot with the 1000 lb. manure application. It would seem, therefore, that an application of 1000 lb. of manure increased the average yield of nitrogen in the crop to the extent of about 6 lb. per acre per

TABLE 1.—Summary of results secured in the continuous growing of corn with a legume green-manure crop, 1908-1926

Year	Legume section							
	No manure		1,000 lb. manure per acre		2,000 lb. manure per acre		4,000 lb. manure per acre	
	Grain	Nitro- gen	Grain	Nitro- gen	Grain	Nitro- gen	Grain	Nitro- gen
	bu.	lb.	bu.	lb.	bu.	lb.	bu.	lb.
1908	39.6	56.4	39.2	59.8	33.1	56.3	37.1	49.1
1909 *	25.0	41.7	34.8	55.1	29.5	49.0	27.7	40.9
1910	28.1	27.8	35.1	33.1	29.7	31.3	35.9	34.0
1911	28.5	43.9	32.1	49.3	26.8	43.3	31.3	47.3
1912	31.6	37.0	36.6	42.3	33.0	37.9	33.0	39.7
1913	33.5	52.4	34.4	54.8	32.1	51.3	33.0	54.6
1914	30.7	42.6	38.6	52.6	36.4	50.8	36.8	50.2
1915	18.6	27.8	22.9	34.9	20.0	38.1	17.5	35.9
1916	35.0	58.1	37.9	60.7	38.2	66.4	42.5	69.1
1917	38.6	44.7	43.6	61.3	41.4	53.3	40.7	58.6
1918	39.9	48.1	41.2	48.5	38.4	46.9	38.4	49.2
1919	30.4	48.8	37.5	57.9	33.7	55.9	33.2	53.1
1920	52.8	60.3	47.1	63.8	48.2	61.8	53.4	68.4
1921	62.0	75.8	72.9	82.4	75.0	86.9	71.4	91.5
1922	44.3	45.2	41.4	45.9	34.3	39.1	54.3	53.9
1923	49.6	67.6	41.5	63.0	45.7	64.4	50.9	72.5
1924	30.1	35.8	38.4	47.9	32.6	40.2	40.4	48.5
1925	56.8	56.3	67.0	70.4	66.6	71.8	68.8	72.1
1926	34.7	41.4	30.1	42.8	32.4	39.3	38.4	46.1
18 yr. Average	37.9	49.1	41.0	55.2	38.7	52.9	41.6	55.6

\* Oats in 1910; omitted from average.

TABLE 1: (Continued).—Summary of results secured in the continuous growing of corn with a non-legume green-manure crop, 1908-1926

Year	Non-legume section							
	No manure		1,000 lb. manure per acre		2,000 lb. manure per acre		4,000 lb. manure per acre	
	Grain	Nitrogen	Grain	Nitrogen	Grain	Nitrogen	Grain	Nitrogen
	bu.	lb.	bu.	lb.	bu.	lb.	bu.	lb.
1908	29.5	48.7	47.2	64.6	42.7	52.7	37.1	49.7
1909	21.4	32.9	26.8	40.5	28.6	43.2	22.3	35.4
1910	21.9	21.1	25.0	25.5	29.7	29.6	26.6	25.9
1911	16.1	26.7	18.8	32.8	32.1	44.1	21.4	38.8
1912	19.6	22.6	28.6	29.3	24.1	30.9	23.2	25.7
1913	20.5	33.4	29.5	50.4	27.2	48.0	22.3	44.4
1914	20.0	30.1	29.6	41.5	34.3	45.1	28.6	39.9
1915	10.0	24.2	16.1	30.3	16.8	28.6	15.0	27.5
1916	18.2	37.4	25.4	45.4	20.7	47.9	28.6	51.6
1917	28.6	34.9	32.1	40.6	35.7	46.8	29.3	42.8
1918	34.9	41.4	38.1	42.9	33.8	43.7	38.5	47.6
1919	22.1	30.2	16.1	26.8	21.1	36.0	24.3	33.2
1920	30.3	40.1	31.7	40.7	32.6	44.1	38.2	55.1
1921	31.1	39.5	34.1	39.4	39.3	44.6	36.7	43.1
1922	27.9	29.3	35.0	37.0	38.6	37.3	31.4	39.9
1923	32.2	40.1	39.8	46.3	42.9	48.0	41.4	53.9
1924	29.3	32.9	38.8	44.7	37.5	44.9	37.5	43.7
1925	47.9	40.3	54.6	48.9	53.8	47.6	57.1	50.6
1926	28.6	25.4	33.3	32.0	37.9	43.9	38.0	40.0
18 yr. average	26.0	33.9	32.0	40.8	33.3	43.2	31.7	42.4

annum, or nearly 108 lb. in the 18 years—more than was applied in the manure and very much more than was presumably utilized from the manure applied. In comparing the nitrogen yields from the legume section with those from the non-legume section, we find that considerable quantities of nitrogen are to be credited to the legumes as compared with the non-legumes. Thus, in the no-manure plots the average yield of nitrogen was 49.1 lb. per acre in the case of the legume section and 33.9 lb. per acre in the non-legume section—a difference of slightly more than 15 lb. per acre. It may be concluded, therefore, that there has been the fixation of considerable quantities of nitrogen in the legume section, the utilization of a part of the nitrogen fixed by the crop and a return from the small quantities of manure nitrogen applied very considerably in excess of that which might be expected under ordinary conditions. The conclusion is therefore warranted that there has been a microbiological effect and that the bacteria present in the manure stimulated the fermentation of the

organic matter in the green manure catch crop, and, finally, the utilization of the nitrogen present in the soil and in the green manure.

### SUMMARY

Experiments have been carried on in the continued growing of Indian corn for 19 years on gravelly loam soil.

Leguminous catch crops, consisting of mixtures of clovers, vetch and alfalfa, on the one hand and non-leguminous catch crops, consisting of rye, have been compared in their effect on the main crop.

Small quantities of farmyard manure, consisting of 1000, 2000 and 4000 lb. per acre, respectively and applied annually, were compared in their effect on the crop.

The leguminous catch crop has increased the yield of both grain and nitrogen as compared with the non-legume catch crop.

There has been a marked inoculating effect from the small quantities of manure applied.

# THE RÔLE OF MYCORRHIZA IN PLANT NUTRITION

M. C. RAYNER

*Bedford College for Women, London, England*

## INTRODUCTION

Whether by reason of its somewhat forbidding title, or because of the unsatisfactory treatment it has received in botanical textbooks, the subject of mycorrhiza has been all but ignored in the literature of soil science. Affecting an immense number of species belonging to numerous and diverse families, and occurring regularly under the most varied conditions of climate and soil, the mycorrhizal habit challenges attention as a factor of great theoretical interest to students of soil biology and significant potential importance to practical growers. Mycorrhiza occurs alike in wild and cultivated plants, in the vegetation of tropical forests as in that within the Arctic Circle, in species from the high alps as in those from the salt marsh.

The history of the subject goes back nearly one hundred years. During the first half of the nineteenth century, more than one observer noticed curious appearances in or upon the roots of vascular plants, and by 1842, Nägeli had identified certain inclusions in the root-cells of *Iris* as fungal growths. When, in 1885, Frank coined the new name *Mycorrhiza* to describe the dual structure formed by the root with its associated mycelium, the phenomenon was already comparatively well-known, especially to foresters.

During the twenty years following Frank's classical researches on trees, the subject attracted many workers, especially in Germany, and its physiological significance aroused some controversy. In certain groups, for example, the Orchids, the relations were noted as specialized and it was observed that in some roots—especially those of Orchids—the intracellular mycelium suffered digestion, so giving rise to soluble products that were apparently absorbed and utilized by the cells of the host.

To botanists these facts were novel and striking, and, although little experimental work was available they served as a basis for various theories of nutrition. On one view, associated especially with the name of Robert Hartig, the root fungi of trees were mischievous parasites hindering root action and conferring no benefit of any kind upon the hosts. On another, the relation was one of mutualism, involving reciprocal benefit and of particular advantage to the vascular partner. These older theories of beneficial symbiosis are especially associated with the names of Frank and

Stahl. Both held that the investing hyphae played an important part in the intake of water and salts. Frank stressed the importance of the soil humus as a source of carbon and nitrogen and he included the formation and intake of suitable compounds of these elements among the services rendered by the root fungi to their hosts. Stahl believed that the fungus partner in mycorrhiza played an important part in maintaining the requisite supplies of mineral salts. He held that the incidence of fungus infection was directly related to the difficulty of procuring inorganic salts and he described all mycorrhiza plants as *mycotrophic* in nutrition.

### REVISED USAGE OF TERM SYMBIOSIS

In this connection, I desire to put forward a plea for a revised usage of the term symbiosis. As at present employed in botanical literature it implies reciprocity with mutual benefit to the participants and it is frequently used in this sense to describe cases for which no experimental data are available. As applied to mycorrhiza plants, for example, it often conveys the impression that the physiological relation between fungus and vascular host is of similar nature in all cases, differing in some fundamental way from that in parasitism generally.

As originally used and defined by de Bary in 1879—the *living together of dissimilar organisms*—the term covers a vast field, the cases included in which obviously require further classification in respect to the mutual relations of the organisms concerned. In this older sense, every mycorrhiza plant is an example of symbiosis. Recognition of the exact nature of the relationship in any given case—whether conferring benefits on one or both partners—must depend, however, upon the accumulation of adequate experimental data, and, until these are provided, must remain to some extent a matter of opinion. The theories of nutrition put forward by Frank and Stahl implied a physiological relation mutually beneficial to fungus and vascular plant. It is the main purpose of the remainder of this paper to estimate the bearing of modern work upon these views.

### MODERN PERIOD OF RESEARCH

What may be called the modern period of research on mycorrhiza began with the present century. It has been marked by recognition of the extraordinary frequency of the phenomenon in nature and by the application of new and more precise methods of experimental research. With this has come realization that the physiology of the relation presents problems closely akin to those of parasitism and pathogenic infection in general, and the conviction that it is only by the use of "pure culture" methods that any substantial increase of knowledge can be gained. Investigations of the kind indicated are laborious and involve the use of a special technique. The isolation of the mycorrhizal fungi, their maintenance in pure culture and the synthesis of fungus and vascular plant all

present difficulties, while evidence of behavior under experimental conditions must be applied with great caution to conditions in Nature.

For these reasons, the bionomics of mycorrhiza and the nutrition of mycotrophic plants are still the subjects of controversy, although enough is known to correlate them with kindred phenomena of parasitism and the parasitic habit and bring them into close touch with the problems presented to the forester and student of plant ecology in the field.

### EXPERIMENTAL EVIDENCE BEARING ON THEORIES

The present occasion offers a suitable opportunity to review the experimental evidence now available and learn how far it lends support to the more or less speculative theories of nutrition put forward by earlier observers. Two observations of a general kind provide indirect evidence in support of the view that there exists in mycorrhiza a reciprocal relation of a beneficial kind: firstly, it is the young and actively absorbing roots which become mycorrhizas; secondly, there is no indication that the root-cells suffer any injury from the extensive invasion by hyphae to which they are subject. Direct evidence bearing on the enquiry has been provided by experimental researches on three groups of plants: Orchids, forest trees and Heaths. It will be convenient to examine these contributions in the order named.

The main facts respecting Orchid mycorrhiza are well-known. The difficulties encountered by Orchid growers in germinating seed, Noël Bernard's discovery of the obligate character of the relation between fungus infection and seedling development, and the association of specific strains of fungi included under the generic name *Rhizoctonia* with individual Orchid species have all become matters of common knowledge. Bernard's conception of the relation between fungus and host in Orchids as one of parasitic attack countered by a mechanism conferring relative immunity upon the host has illuminated many aspects of the problem, but it does not provide an explanation of the most characteristic features of the association, namely, the obligate nature of the relation with seedling development and the association of specific strains of mycelium with particular Orchids over wide geographical areas.

With respect to nutrition it can hardly be doubted that the balanced relation observable in living Orchids has been evolved from one following parasitic attack upon the roots by soil fungi, although the evolution of an obligate symbiotic relation of this kind offers an unusually difficult problem to the student of heredity. It was in Orchids that the true nature of root infection was first clearly recognized and the physiology of the symbiotic relationship from the first attracted much attention.

Admitting the cogency of all the traditional arguments based on the utilization of organic humus compounds by the fungi and the wholesale digestion of mycelium that takes place subsequently in the root-cells,

there remains much that is puzzling in the relation of fungus and host in this group of plants.

An outstanding difficulty in attributing a beneficent rôle to the mycelium is the relatively scanty development of hyphae on the external surface of the roots. In the case of calcicolous species, moreover, it is not clear that a significant increase of nutrient substance can be obtained from organic sources in the soil. On the other hand, Orchids show a characteristic intermittent type of infection which, in itself, involves a recurrent "tapping" of the contents of hyphae in direct contact with the soil humus, while the existence of a large group of non-chlorophyllous forms in itself provides a tempting argument in favor of the view that the endophytic fungi play an important part in the nutrition of both green and non-green species. In opposition to the commonly accepted view, a claim has been put forward recently for fixation of atmospheric nitrogen by the root-fungus of *Neottia*, a characteristic non-chlorophyllous humus species. In general, it appears to me fairly certain that a better understanding of nutritional problems in Orchids with special reference to mycorrhiza must await more complete information as to the exact mode of nutrition of the non-green forms as of other so-called "saprophytic" species growing in humus soils. Modern research methods have not yet proved adequate to the task of germinating seeds of such species, a necessary preliminary to the study of these curious and interesting plants under "pure culture" conditions.

In view of the necessity for restricting our observations on mycorrhiza to such facts as can be directly correlated with soil conditions, it is only possible to note in passing the biological interest and practical importance of the aspects of Orchid mycorrhiza such as the application of symbiotic and asymbiotic methods to facilitate germination of seed in horticultural practice. The use of asymbiotic methods has a special interest in this country in view of Knudson's important researches on the subject. There is, however, no evidence that asymbiotic germination ever occurs in Nature.

Much light has recently been thrown on the bionomics of tree mycorrhiza by the researches of the Swedish botanist, Melin, and certain facts may be regarded as well established. In Sweden a number of Hymenomyces common in woods have been identified as the specific root-fungi of Pine, Spruce and other trees. These forms belong to saprophytic genera and there is no evidence of parasitism of the ordinary kind. They show specialization to the symbiotic habit, and it is possible that some of them are obligate symbionts, unable to complete their development except in association with the roots of their hosts. Neither true parasites nor true saprophytes physiologically, the name "*symbiophiles*" has been suggested for them.

It has been proved experimentally that normal mycorrhiza is developed

only in a suitable rooting medium and that its formation is markedly sensitive to changes in the reaction of the medium, and the presence of unsuitable nutrients or toxic substances such as those produced by heat sterilization. There is no evidence that the early stages of seedling development are bound up with infection and, in general, the formation of mycorrhiza by trees appears to be a phenomenon of reciprocal character, conditioned by the physiological state of the symbionts. With respect to nutrition, the evidence indicates that absorption of inorganic salts is carried on in raw humus soils rather more efficiently by infected than by uninfected roots. The most significant results, however, relate to nitrogenous metabolism.

There is at present no evidence for fixation of atmospheric nitrogen by any of the known root-fungi of trees. On the other hand, it has been shown that they utilize such organic compounds of nitrogen as are normally present in humus much more efficiently than do seedlings of their hosts. That the latter profit is also clear from the experiments with infected and uninfected seedlings. It may be concluded that mycorrhiza possesses a vital significance for trees and other plants growing in raw humus soils.

In northern Europe tree mycorrhiza is typically developed under such conditions and there is reason to believe that the soil reaction and the character of the organic compounds are important factors in promoting its healthy development and functioning. It is of interest to note that these conclusions are supported by Falck's observations on the nutritive significance of mycorrhiza in acid woodland soils in Germany. There can be little doubt that further experiment will permit their extension to all trees growing in such soils and showing a similar mycorrhizal habit. Turning to Ericales, the group in which my own interest has centered, we must note the edaphic peculiarities of many of the species. *Calluna vulgaris* and many of its allies are characteristic and abundant members of the vegetation of humus soils—moorland, heath and woodland—in various regions.

Although an obligate association with specific endophytes is a feature common to these plants as to Orchids, the mycorrhiza of Heaths shows many points of resemblance with that of trees. Indeed, in my opinion, the interest of mycorrhiza to students of soil conditions at present centres about the nutritive relations of forest trees and of certain Ericaceous species with their respective root fungi.

In *Calluna* and certain of its allies an obligate symbiotic relation unique of its kind has been revealed by experiment. In these plants, the mycelium which profusely infects the young roots ramifies throughout the tissues of the vegetative shoots, flowers and fruits, infecting the seed coats of the developing seeds which are eventually shed bearing with them the hyphae of their fungal symbionts. Seeds, superficially sterilized and



sown under aseptic conditions, germinate freely, but the resulting seedlings are incapable of forming roots or of advancing beyond the earliest stages of development.

The obligate character of the association is probably confined to the seedling phase and it is not at present certain—either in Orchids or Heaths—that it extends to the adult, although infection of the roots follows inevitably upon that of the seedling tissues. Both in *Calluna* and in trees it has been shown that the formation of functional mycorrhiza is conditioned by the character of the rooting medium, and there is evidence that reduction or suppression of mycorrhiza formation in correlation with external conditions is associated with changes in the metabolic activities of the root cells. In Heaths as in Orchids, there is wholesale digestion of the intracellular mycelium indicating that—as in trees—the possession of mycorrhiza, enables the vascular hosts to draw indirectly upon the organic reserves locked up in humus. In Ericaceae the relation is more specialized than in trees, and there is good evidence that the capacity to draw indirectly upon the organic compounds of humus is supplemented by the ability of the root-fungi to utilize atmospheric nitrogen.

It is significant that Ericales, like Orchidaceae, includes a number of so-called humus “saprophytes” entirely deprived of Chlorophyll and it is tempting to regard these, in both cases as the end terms of a series of adaptations following upon increased dependence upon the symbiotic habit. Assuming the possibility of independent development, it is possible that plants normally subject to mycorrhiza might thrive better under the relatively favorable conditions inseparable from “pure culture” experiments if protected from infection, whereas in individuals of the same species exposed to competition in the field, the possession of mycorrhiza may have assumed critical importance. Great caution must therefore be exercised in extending conclusions deduced from “sheltered” experimental cultures to plants growing in Nature.

The experimental results I have cited point especially to the significance of mycorrhiza in relation to nitrogenous metabolism. We know that the severity of the “struggle for existence” among plants not uncommonly centers about what is known as the “nitrogen problem.” On *a priori* grounds it is not unreasonable to believe that the mycorrhizal habit so common in vascular plants, as well as the corresponding fungus infection in Liverworts are, like the root-nodules of legumes, but another manifestation of the urgency of this problem. In two of the three groups of mycorrhiza plants just considered, experimental research has provided corroborative evidence that this is actually the case.

Outside these specialized groups there is yet little experimental data available. Modern work has emphasized the extraordinary prevalence of the habit and indicated that the root fungi of a majority of Flowering

plants and Vascular Cryptogams belong to a common group of soil fungi. On the view put forward recently by an Italian observer, Peyronel, root-infection is frequently of a dual character, involving primary infection by a fungus of the usual kind followed by secondary infection by one of the type associated with Orchids. Whether this "double infection" is of biological significance is at present unknown. It is possible only to mention in passing the theory of tuberization originally put forward by Bernard and recently elaborated and extended by his colleagues at the Pasteur Institute. This theory relates tuberization and indeed the perennial habit in herbaceous plants generally, directly to root infection by soil fungi. The evidence is suggestive but at present hardly adequate to carry the weight of such a far-reaching hypothesis.

If we now enquire the bearing of these modern researches on the older theories of beneficial symbiosis so strongly urged by Frank and Stahl, it may safely be concluded, I think, that recent investigations by means of "pure cultures" provide firm support for the belief that the possession of mycorrhiza is frequently of service to the vascular hosts, the nature and degree of the benefit depending upon the physical environment of the roots and the exact nature of the biological relations in any given case. The application of the results to natural conditions raises problems of great importance, especially to foresters, and opens up a new and promising field of research.

A word as to the systematic position and distribution of those mycorrhizal fungi whose identity has been established. Forms definitely associated with tree mycorrhiza belong to that great group of higher Basidiomycetes, especially Hymenomycetes, whose fruit bodies are so prominent a feature of woods. One of the most interesting contributions recently made to the subject was the proof supplied by Melin that a number of species of *Boletus* common in Coniferous woods were the root symbionts of Pine, Spruce and Larch. Mycelium of all these forms must be widely distributed in woodland soils and research is needed to determine whether steps should be taken to ensure their presence in newly afforested areas.

The root-fungi of Orchids are generally recognized as belonging to a common group, sometimes included in the well-known form-genus *Rhizoctonia*, sometimes placed in a new genus, *Orcheomyces*. The mycelium must be locally present about the roots of Orchids but nothing is known as to its general distribution in soil or its relationship, if any, with other soil fungi included in the genus *Rhizoctonia*. The mycorrhizal fungi of *Calluna* and other ericaceous species belong to the genus *Phoma*. Mycelium of the various strains must be locally distributed in heath and moorland soils carrying ericaceous vegetation, but there is no certain knowledge of their wider distribution.

Outside these specialized groups, the fungi responsible for mycorrhiza

formation still await identification. The mycelium almost invariably present is of uniform type with characteristic structural features, and resembles that known to occur commonly in decaying plant detritus. It has hitherto resisted all attempts at isolation. Speculation attributes it to a member of the Lower Fungi, but nothing is known with certainty as to the systematic position of this widely distributed soil species.

In the necessarily limited time at my disposal, it is impossible to do more than give a mere indication of the practical interest and potential importance of the results already achieved. I can only express the hope that the same reason may be accepted as a partial excuse for the inadequate manner in which I have treated this most fascinating aspect of soil biology.

#### LITERATURE CITED

- (1) Falck, R. 1923. *Mykologische Untersuchungen und Berichte*. 2: Cassel.
- (2) Melin, E. 1923. *Experimentelle Untersuchungen über die Konstitution und Ökologie der Mykorrhizen von *Pinus silvestris* L. und *Picea Abies* L. Karst.* *Mykologische Untersuchungen* (Falck). 2: Cassel.
- (3) ———. 1925. *Untersuchungen über die Bedeutung der Baum-mykorrhiza.* Jena.
- (4) Rayner, M. C. 1925. The nutrition of Mycorrhiza plants. *The British Journal of Experimental Biology*. Vol. 2, 1925.

# SOME CONSIDERATIONS ON METHODS OF SOIL BIOLOGY

A. BONAZZI

*Chaparra Agricultural Experiment Station, Cuba*

The fact that many of the concepts, once held as axioms in soil biology, are now passing to the realm of unproved hypotheses, stands as an indication that some fundamentally erroneous assumption is at the basis of the difficulties we encounter in attempting to correlate fertility with soil biological activities, as they are known to us today. Since soil biology is essentially a science of observation and experimentation, we are justified in asking whether this, or these, erroneous assumptions are to be found in the present formulation of our concepts, or in the establishment of our present methods.

The present tendencies toward a standardization of our methods of work would seem to point to the fact that these are the phase of our science that is based on rather unsafe foundation. However, careful consideration of the matter leads, on the contrary, to the realization that our present concepts relative to the nature and function of soil biological activities have often assumed an homocentric character irrespective of the natural requirements and adaptabilities of the organisms that form the objects of our study.

It is, therefore, with the aim of presenting a few novel conceptions that the present contribution is made. Without desiring to appear to minimize the value and importance of the careful and conscientious work, and the achievements of our predecessors, the author thinks that, with Winogradsky, we can summarize the situation thus:

“Les notions que nous possédons sur les phénomènes microbiennes dont le sol est la siège, ne sont, que très fragmentaires et manquent de précision. Au bout d'un trentain d'années de travail on a réussi, il est vrai, à isoler un certain nombre de microbes du sol et à reproduire en culture pure la majorité des processus qui intéressent la science agronomique . . . mais quelque intérêt que puissent présenter ces études physiologiques nous n'y trouvons que peu de renseignements sur les espèces que peuplent les différentes terres, ni sur leurs fonctions, telle qu'elles s'exercent dans la nature, ni, surtout, sur les rapports biologiques qui y règnent et qui règlent la succession des processus et le sort de ces espèces que nous avons tous raison de nous figurer en lutte incessante pour s'approprier la matière énergétique . . .”

When we examine a soil from a biological standpoint, we are often too prone to forget that, by virtue of its physical and chemical constitution, the soil presents a given environment to its biota and that, therefore, the

latter is continually in a state of dynamic equilibrium therein. Some of the individual members of this complex biota, we can separate and study by our selective-culture methods, but the majority of the component organisms of this biota have, so far, been out of our reach, and this, not because we have not isolated plenty of organisms from the soil, but because we have often isolated and studied forms which were not typically edaphic forms. It is for this reason that we are not yet in a position to determine, by our present methods, the shiftings of the above mentioned equilibrium that result as a response to slight changes in physical and chemical environment.

It is the contention of the present author that by maintaining a closer correlation between these various factors and following the biological changes of activity coincident with arbitrary established changes in environment it is possible to follow the shift in this equilibrium and make a step in the right direction in the interpretation of the part that the soil biota plays in the phenomena of fertility of a soil.

The author had occasion to study the biological behavior of three plots of the Ohio Agricultural Experiment Station five year Rotation Experiment in Wooster, Ohio. The treatments summarized below were applied to these plots for a period of over 31 years, after which time the condition of the soils, with respect to their acidity, was found to be as follows:

TABLE 1.—*Hydrogen ion concentration of five year rotation*

No. of plot	Fertilizer treatment	Liming	Acidity expressed as pH
18a	Manure	Limed end	7.66
18b	Do	Unlimed end	6.73
22a	Untreated	Limed end	7.87
22b	Do	Unlimed end	4.99
24a	Ammonium Sulfate <sup>a</sup>	Limed end	7.87
24b	Do	Unlimed end	4.99

<sup>a</sup> A complete fertilizer composed of acid phosphate, muriate of potash and nitrogen as ammonium sulfate.

In view of the great differences in acidity between the limed and unlimed portions of the same plot, dilution platings were made in media similar to that recommended by Thornton, which, however, had been adjusted to various degrees of acidity. The results obtained on the sixth day of incubation are hereby summarized and recalculated on the basis of the percentages they constitute of the total number of colonies found at a pH of 5 (Table 2 and Fig. 1).

TABLE 2.—*Number of bacteria per gram of soil and relation between the numbers in the limed and unlimed*

Reaction pH of plating medium	Manure		$\frac{L}{U}$	Check		$\frac{L}{U}$	Ammonium Sulfate		$\frac{L}{U}$
	Limed	Unlimed		Limed	Unlimed		Limed	Unlimed	
pH 7	605,736	225,600	2.68	121,250	47,960	2.50	172,200	140,450	
6	1,071,600	415,600	2.56	124,650	32,370	3.82	303,900	348,100	
5	2,011,224	751,600	2.68	252,650	279,400	0.91	439,000	548,550	
4	41,736	142,450	0.29	3,399	27,575	0.12	52,900	123,650	

Without entering into a discussion of the practical application of these results, we shall only take up the matter of the better understanding they afford us of the biological state of the soils studied.

A comparison of the actual numbers found to grow at any arbitrary degree of acidity show in all cases that it is possible to only half interpret the values obtained. It is only, when the data based on percentage is presented in a graphic form, that the full bearing of the results is gathered. The unlimed soils in all cases have a greater "acidophylous" and a lesser "alkaliphylous" flora than the limed soils. It is, thus, by the artificial shifting of the "biotic equilibrium" in the soils studied, utilizing for the purpose an outstanding difference in the physico-chemical soil complex, that this light is gained, and not by using an arbitrary set standard procedure.

The following curves, indicative of the shift in bacterial equilibrium, lead us to look for an adaptation of the soil biota to its environment. The fact, however, that this adaptation may follow two paths (a) one of species adaptation by selection of more acidophylous species as the acidity of the soil waters increases and (b) one of interspecific adjustment by an adaptation of the same forms through a slow but deep change in their physiology, is not the province of this paper, and is now being carefully studied in tropical soils. It is sufficient to show here that the establishment of the "biotic-equilibrium" curves is of far greater import than the simple use of standard arbitrary methods.

In view of the above results, we are justified in studying whether the adaptation of bacterial forms to greater concentration of free hydrogen ions in the soil waters is accompanied by a greater activity of this soil biota in more acid media. That this is what actually happens is shown by the graphical presentation, given below, of the results obtained in a study of the amino acids developed in solutions of casein of different degrees of acidity inoculated with equal weights of the soils mentioned above (fig. 2).

It may be mentioned here that the formation of ammonia, even though quantitatively followed failed altogether to yield indications of value.

Still more interesting results are obtained when, by allowing the soil biota itself to shift its own equilibrium, through the fermentation of an

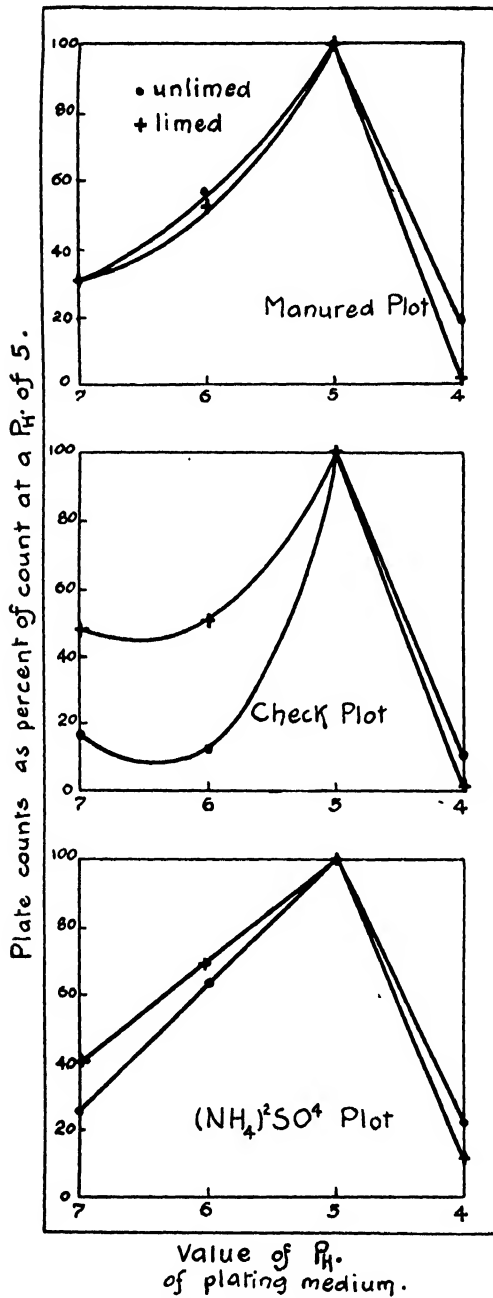


FIGURE 1.—Relative distribution of bacteria in media of different acidity, referred to the values obtained at pH 5 as 100

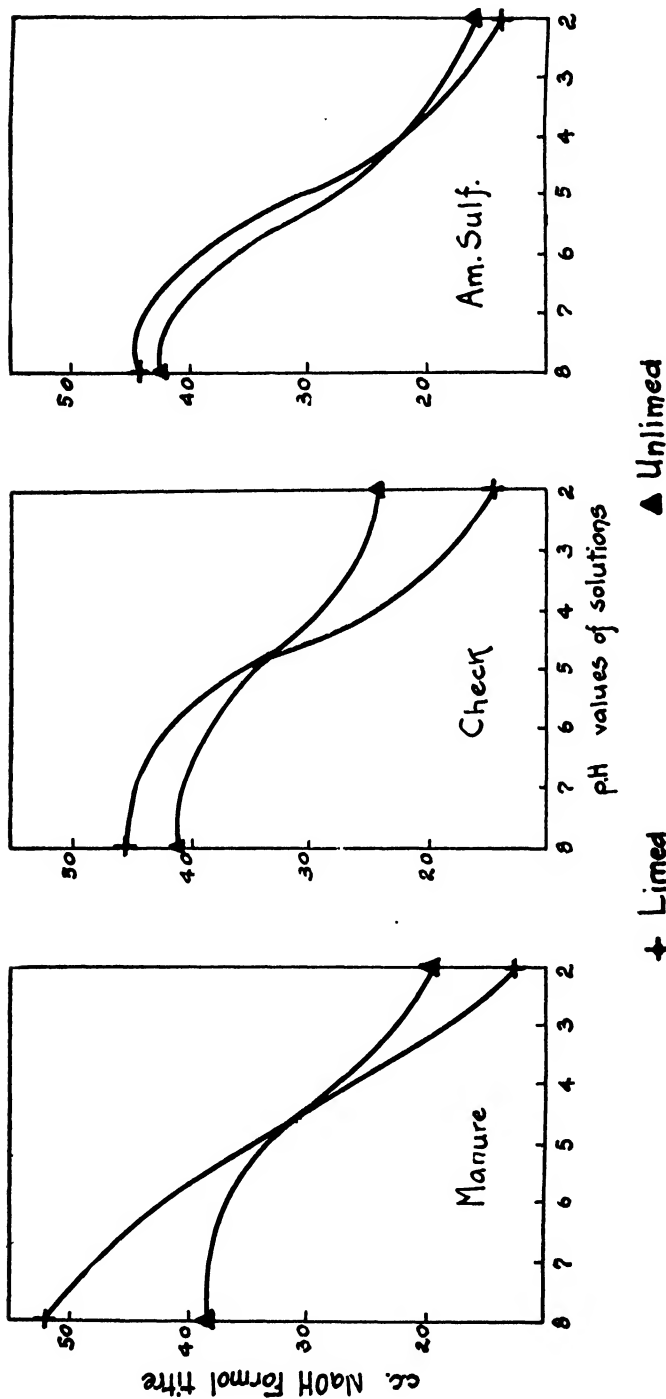


FIGURE 2.—Amino acids developed in solutions of different degrees of acidity when inoculated with soils differently treated



added carbohydrate, the results are plotted as mentioned above. Table 2 and Fig. 3 speak for themselves. In this case a solution of native egg albumine, alone or with the addition of 1 per cent glucose, was inoculated with equal weights of the soils mentioned above.

TABLE 2.—Formol titer on 100 cc. of solution expressed in cc. 0.2 N NaOH

Treatment of plot	No. of culture	Days of incubation				NH <sub>3</sub> -N mg. in 100 cc.	Acidity cc. 0.1N acid	Indol reaction qualitative
		0	3	7	10			
No sugar cultures								
Manure limed	1	0.00	6.33	17.32	15.40	13.78	5.85	+
Manure unlimed	2	0.00	4.46	13.92	23.00	18.58	5.15	+
Check limed	3	0.00	2.06	13.99	18.25	18.90	7.15	+
Check unlimed	4	0.00	1.93	14.19	17.25	14.41	5.40	+
Amm. Sul. limed	5	0.00	4.46	19.31	23.15	15.67	4.60	0
Amm. Sul. unlimed	6	1.53	2.26	19.98	23.90	14.05	6.75	0
Sugar cultures								
Manure limed	1s	0.00	0.00	2.13	3.15	0.00	38.00	0
Manure unlimed	2s	0.00	0.60	1.27	2.30	0.00	30.85	0
Check limed	3s	0.00	0.73	0.93	2.05	0.00	35.15	0
Check unlimed	4s	0.00	1.00	9.12	17.35	0.00	16.70	+
Amm. Sul. limed	5s	0.00	0.87	7.66	16.65	0.00	17.80	0
Amm. Sul. unlimed	6s	0.00	0.80	16.05	27.00	0.00	17.55	+

These curves and table are of paramount importance insofar as they show: (a) ammonia determinations lead to very questionable results, and (b) the soil biota establishes by means of its own activities its own limiting conditions shifting thereby its equilibrium to a considerable extent.

It may be mentioned here that the outstanding differences between these soils are of a varied character including as they do different degrees of acidity, a heavy manuring with stable manure, a chemical fertilization with nitrogen in the form of ammonium sulfate and no treatment whatsoever. The results given above, even though emphasizing in the present case the acidity gradient of biological activities also give a clear idea of the status of these soils when they are compared one to another with respect to the other characters in which they differ. For lack of space it shall only be possible to mention here that the soils studied, even though originally of the same composition, differed, after the 31 years of treatment to which they have been subjected, profoundly in degree of aeration, water retention, tilth and chemical composition. The data summarized above shed considerable light on the effects which these differences in physico-chemical state have on the biotic equilibrium of the soils themselves.

By allowing the breakdown of a native protein to take place in the presence of an easily fermented sugar, it is possible to measure the protective influence that the carbohydrate has on the nitrogen com-

pounds. It is thereby possible to obtain a measure of the proteoclastic activities in the soil and an indication of that condition in the cultures which, reached by a mechanism inherent to the specific flora of that soil, does not permit a breakdown of the added protein.

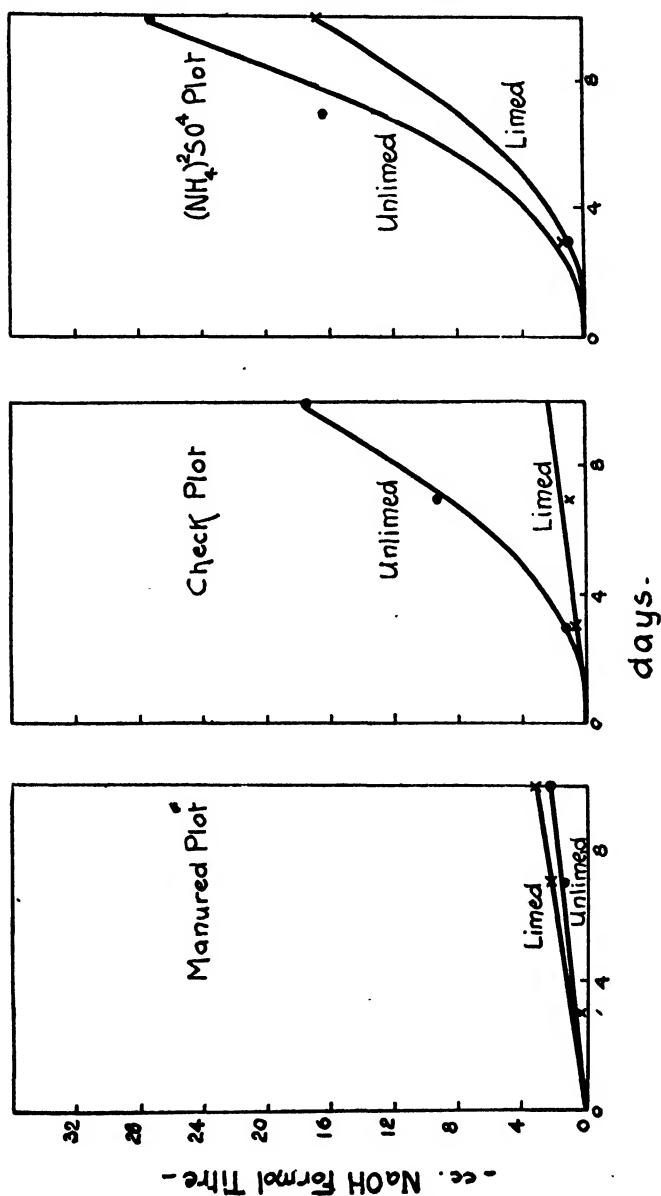


FIGURE 3.—Amino acids developed in native egg albumine in presence of glucose

That this value has a practical bearing is shown by the fact that, in Table 2, through the fermentation of the carbohydrate, the solutions inoculated with the manured soils reached a limiting point so soon that

practically no proteolysis took place, indicating a greater carbohydrate attack than that to be found in the other soils. This fact, coincident with the great differences in bacterial content between the limed and unlimed portions of the manured plot, shows that the yearly additions of bacterial cells with the manure are not of a type to effect the protein cleavage at the limiting point which this same flora can reach by natural processes.

The fact that the unlimed portion of the manured plot did not differ greatly from the limed, as it did in the other two plots, is to be correlated with the great buffer action of the organic matter added with the manure an action which, lacking in the other two cases, keeps the pH value of the soil from two extremities of the manured soil to a very equal level.

In the other two soils, where there is no periodical renewal of the flora and where processes of adaptation have been at work for a long time, the differences between the limed and unlimed ends are sharply defined. The limiting point may be reached more or less speedily according to whether the soil has or has not a numerous flora. The nature of the nitrogenous compounds on which the flora is made to act also has a bearing as to whether the degree of acidity reached during the carbohydrate fermentation is or is not a natural limiting factor in protein cleavage. In fact on long standing, peptone may be attacked in a different manner by the limed and unlimed manured soils, as is shown in Table 3 and Fig. 4, where the values are given up to 13 days of incubation of a Witte peptone solution inoculated with the soils mentioned above. Here, up to the seventh day of incubation, the behavior of the manured soils is very similar to that of these same soils in presence of albumine.

TABLE 3.—Formol titers in peptone cultures—cc. 0.2 N NaOH in 100 cc. of solution

Soil	No. culture	No. of days				Ammonia nitrogen at end of 13 days mg. N	Indol	Biuret
		0	3	7	13			
Manure limed	1	6.42	17.43	54.34	59.47	33.49		0
Manure	2	6.60	17.79	66.93	58.20	67.06		0
Check	3	6.30	12.77	64.40	58.87	69.06		0
Check limed	4	6.60	14.85	67.13	37.22	67.49		0
Manure limed	1s	6.60	6.50	9.52	10.12	2.86	0	
Manure	2s	6.78	6.23	9.66	35.36	28.13		
Check	3s	6.60	6.29	14.32	24.10	19.71		
Check limed	4s	6.50	6.33	8.52	12.99	3.72	0	

Another example emphasizing the value of the establishment of curves of activity is afforded by the following graphs (fig. 5) picturing the nitrifying capacity of four types of soil. By adding, to various samples of the same soil, increasing amounts of precipitated calcium carbonate,

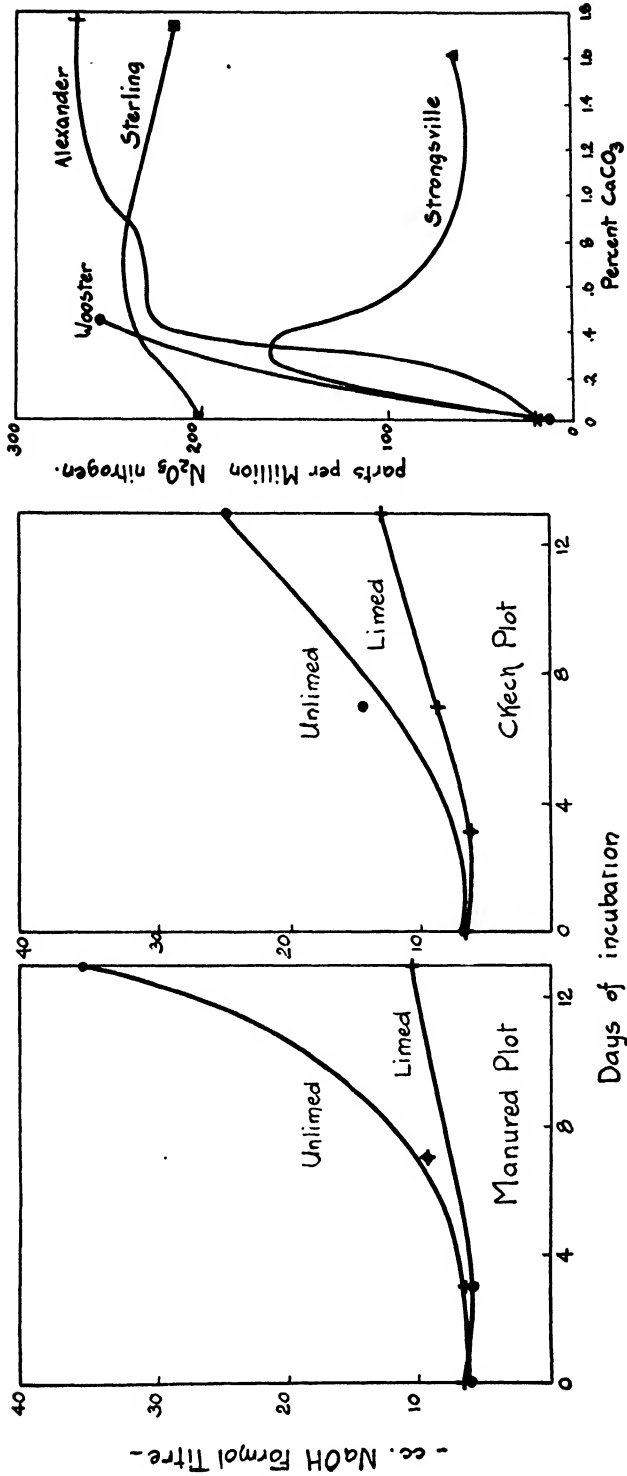


FIGURE 4.—Amino acid formation in peptone solutions in presence of glucose

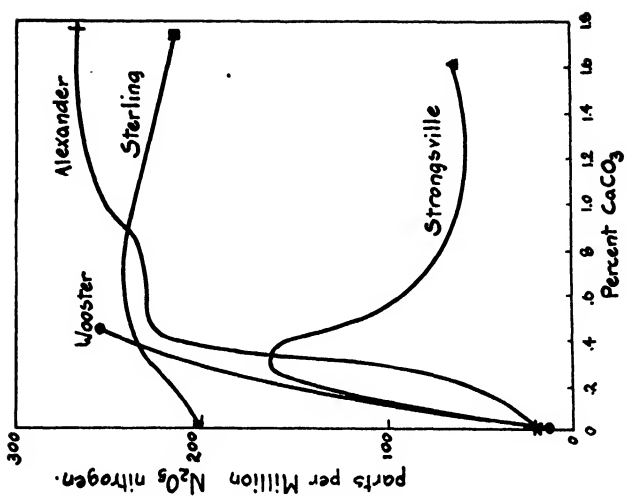


FIGURE 5.—Nitrification in different soil types brought to contain different quantities of calcium carbonate

a gradient was established which served to shift the biotic equilibrium therein. It is very evident here that the choice of any arbitrary figure, obtained along the path of the curves secured, in this experiment, far undervalues the potential capacities of the soils under examination.

In this case, the comparison is not only one of acidity, but of soil type, and each curve characterizes perfectly the soil it represents.

Similar curves can be established relative to any other function or outstanding character of a soil, or of various soils, offering, as has been stated before, a method for the appraisal of the fertility potentialities of the soils themselves.

The present is not a syllabus of standard methods for the study of soil biological activities, and it is for this reason that details of manipulation are not given. The object of this contribution is only to draw the attention of workers in this field to the great possibilities of interpretation that these methods afford, and a plea against an over-standardization of methods that should, undoubtedly, stifle developments in the field.

#### LITERATURE CITED

- (1) Conn, H. J. 1927. Soil flora studies. III Spore-forming bacteria in soil. New York (Geneva) Agr. Expt. Sta. Tech. Bul. 58.
- (2) ———. 1917. Soil flora studies. IV Non-spore-forming bacteria in soils Ibid. 59.
- (3) ———. 1918. The microscopic study of bacteria and Fungi in soil. Ibid. 64.
- (4) ———. 1919. Ammonification of manure in soil. II Taxonomic study of two important soil ammonifiers. Ibid. 67.
- (5) ———. 1925. Soil flora studies. VI The punctiform-colony-forming bacteria in soil. Ibid. 115.
- (6) Gainey, P. L. 1917. The significance of nitrification as a factor in soil fertility. Soil Sci. 3: 399.
- (7) Halversen, W. V. 1925. A study of the biological activities in certain acid soils. Oregon Agr. Expt. Sta. Bull. 211.
- (8) Russell, E. G. 1921. Les Microorganismes du sol dans leurs rapports avec la plante. Position actuelle du problème. Ann. Sci. Agr. 38<sup>e</sup>, 6<sup>e</sup> ser. 49-68.
- (9) ———. The microorganic population of the soil Chapter V. Rothamsted Monographs on Agr. Sci.
- (10) Temple, J. C. 1919. The value of ammonification tests. Georgia Agri. Expt. Sta. Bull. 126.
- (11) Thronton, H. G. 1922. The development of a standardized agar medium for counting soil bacteria with especial regard to the repression of spreading colonies. Ann. Applied Biol. 9: 241.
- (12) Winogradsky, S. 1924. La méthode directe dans l'étude microbiologique du sol. Chimie et Industrie 11, No. 2.
- (13) ———. 1924. Sur la microflore autochtone de la terre arable.
- (14) ———. 1924. Sur la méthode de la microbiologie du sol. Actes 4<sup>e</sup> Conf. Intern. Pedol. Vol. 248.

# A NEW GENETIC PHYSICO-CHEMICAL THEORY OF THE FORMATION OF HUMUS, PEAT AND COAL

## THE RÔLE AND SIGNIFICANCE OF BIOLOGICAL FACTORS IN THESE PROCESSES <sup>1</sup>

J. ZOLCINSKI

*Polytechnic Institute of Lwow, Dublany pres Lwow, Lemberg, Poland*

The formation of humus in general (eremacausis putrificatio), putrefaction, general disintegration with the formation of peat, starting with the first stages of browning, is a physico-chemical and not a biological (bacteriological) process as has hitherto been maintained. During these processes of humification of organic plant and animal substances there are formed, in parallel reactions, hydrogen peroxide and benzenoid and cyclic compounds in general which, with the hydrogen peroxide, have an antiseptic action. The antiseptic properties of peat have been known for a long time.

The influence of sunlight, not only thermal but radiant, takes part in the formation of humus on the surface of the earth and in a certain depth of the soil. In the deeper layers pressure and temperature function as physico-chemical factors.

The process of natural decomposition of organic materials (plant and animal) in the course of time is subject to the same fundamental law of decomposition as are rocks and minerals, by which complex silicates are metamorphosed into simpler and more stable compounds. All organic substances obey the same law during the decomposition of different organic materials (plant and animal); they lose the elements of water, that is hydrogen, and oxygen, becoming richer in carbon and nitrogen (in the first stages of humus and peat) and become more condensed and more stable, under the physiographical conditions prevailing in the terrestrial globe. Finally this process of simplification and stabilization of organic compounds takes the form of coal, that is to say, of brown coal (lignite), black coal, anthracite, and finally graphite. During these stages of decomposition of organic matter we behold not only a decrease in the water elements, hydrogen and oxygen, but also of nitrogen and of ash, and simultaneously a great increase in carbon in its pure forms, as for example in graphite. These combinations represent the end-point of stabilization, for, as is known, carbon belongs to the most stable and

<sup>1</sup> An abstract of the original article.—Ed. note.

condensed elements under the physiographical conditions prevailing on the terrestrial globe.

Moreover, physico-chemical humification proceeds more energetically, the more double bonds there are present in the chemical structure of the material which is decomposed. Hence, the author affirms that all the biological factors, utilizing the chemical energy accumulated in organic matter, have for their end, as a final result, the transformation of organic substances into chemical compounds with double bonds, which are suited to physico-chemical humification. In this manner complete mineralization of the organic matter is assured. By this natural process there is obtained the accumulations of humus, of peat and of fossil organic matter; it is in accord with the fundamental law of economy of nature to accumulate energy in a potential form.

Benzenoid organic substances, with and without nitrogen in their constitution, such as hydroquinone, tannin, pyrocatechin, quinone, resorcin, gallic acid, tyrosine and albumin, are humified not only under the influence of sunlight and in the dark (aromatic substances not containing nitrogen), but also under the influence of very weak light, as for example from electric lamps ("Osram Nitra" of 200 candlepower). Hydrogen peroxide is formed in the solutions of the substances mentioned, simultaneously with humification. Aliphatic compounds, not possessing double bonds, are not humified and do not form hydrogen peroxide under the same conditions.

Professor Palladine maintains, as do I, that the dark brown or brownish-black coloration in the dead tissues of plants under anerobic as well as aerobic conditions is a physico-chemical process, but respiratory, effected under the influence of oxidizing enzymes, called oxidases and peroxidases. The latter act exclusively upon aromatic compounds, called by the same author chromogenic respiratory pigments. The author employs the term chromogen in speaking of living plants in which the chromogens may not be manifest and appear only after the death of the tissues; the oxidases do not oxidize aliphatic substances; oxidation is effected only in the presence of the aromatic substance, as that which transpires oxygen, or after re-synthesis of the aliphatic substance in the same plant into an aromatic compound. Oxidation in plants never appears without the presence of chromogens. The identity of pigmentation and humification is indubitable. Since the mother substance is the same, the aromatic substances and the energy of formation of the dark substances are also the same. For aromatic para-compounds it is the highest, for ortho-compounds it is lower, and for meta-compounds it is the lowest (Bertrand). The identity of the processes of humification and pigmentation will be confirmed by the identity of the absorption spectra of these substances.

Conforming to our conclusions and to our assertions that nature tends toward the building up of aromatic substances (Par. 4), Professor Pal-

ladine has experimentally established the same tendency in plants and he has demonstrated that the first product of the assimilation of carbon by plants is glucose. Glucose is the parent substance which serves for the synthesis of aromatic compounds in plants; in the plant cell, just as during dry distillation, the benzene ring is formed from glucose. This genesis is approved by Waage (of glucose) and by Hazura and Benedict (of carbohydrates). These authors emphasize the fact that phloroglucine ( $C_6H_3(OH)_3$  1, 3, 5) is very widely distributed in plants.

Our conclusions demonstrate that the energy of decomposition of crystalline organic substances, under the influence of light and in darkness, with the liberation of hydrogen peroxide (*in statu nascendi*) and with the formation of colloidal humus bodies, is exerted most vigorously in the case of aromatic compounds with OH in the para-position, more weakly where it is in the ortho-position, and most weakly where the meta-position is involved; this is in accord with the conclusions of Bertrand relative to laccase (Par. 6).

The gradual increase in the nitrogen content of peat, with depth, is explained by the experiments of Professor Palladine, which indicate that in anerobic conditions (in hydrogen) oxidation (respiration with the aid of respiratory pigments) takes place at the expense of oxygen from carbohydrates and not from the albumin of plants.

Under the influence of pressure and temperature, there are formed gradually, in the layers of the coal deposits, double-bonded compounds of cyclic structure, containing benzene and other rings (from benzene, naphthalene and anthracene up to leucasene and others). These compounds, under the conditions cited above, in proportion as they are formed, are decomposed by degrees, lose the water elements, hydrogen and oxygen, as well as nitrogen, and gradually become higher in carbon. The process which I postulate for the dentrification of coal takes place as follows: The nitrogenous organic compounds of the coal are decomposed by hydrogen peroxide to amides and finally ammonia (Experiments of Mr. J. Effron with albumins and amides, as well as those of Prof. A. G. Dojarenko with humus, p. 9. *renv. Nr. 18a*); the latter ( $NH_3$ ) is then oxidized to nitrites,  $NO_2$ , and then to nitrates,  $NO_3$ . Professor A. N. Bach demonstrates by his experiments, the formation of nitrites from amidized substances in plant extracts under the influence of oxidases, and Maze reports likewise a direct oxidation of albuminous substances to nitrites, during the respiratory process. Thus is proved the facility of oxidation of nitrogenous compounds and ammonia. The bases of the ash serve as cations for the nitrites and nitrates which are formed in the coal. Being very soluble they are finally leached out of the coal.

Crystalline aromatic substances, in dilute solutions, behave in the dark and especially in light, just as the enzymes, oxidases and peroxidases; for they liberate hydrogen peroxide which has an oxidizing action in *statu*



nascendi. They are decomposed in light, in a monomolecular reaction, forming active oxygen (A. Tian). This process leads (a) to a recognition of the nature of the enzymes, oxidases and peroxidases, of their origin and their chemical action, and (b) serves also for the production of artificial enzymes. Artificial humus, as a physico-chemical catalyzer which in light energetically oxidizes ammonia, to nitrites, and nitrates, might serve, according to my experiments, for the artificial production of the enzymes, oxidases and peroxidases. In nature they are only transitory links in the chain of changes which leads to solid and stable forms.

Levulose (1 double bond) is humified with difficulty in sunlight. Formaldehyde appears in the levulose solution in 140 days at Moscow (V-VIII). Here is an important fact. The reduction of the sugar to formaldehyde (perhaps under the influence of hydrogen peroxide) demonstrates that the theory of the formation of sugar from formaldehyde (Butlerow 1861 and Low 1886) is entirely valid, since levulose may be partially reduced to formaldehyde under the influence of sunlight.

Urea (1 double bond) is decomposed, not only under the influence of sunlight, which takes place very rapidly, but also under the influence of a very weak electric lamp (incandescent electric lamp, "Osram Nitra" of 200 candlepower). In the latter case the decomposition proceeds very slowly and there is no humification, even in the sunlight. The nitrates which are formed furnish us a very clear and interesting proof of photo-chemical denitrification.

Considering all the facts and all the results cited above, we reach the following general conclusion: My genetic and physico-chemical theory of the formation of humus, peat and coal is in accord with facts observed in nature and with the results of actual laboratory experiments.

# PLANT RESIDUES IN TROPICAL SOILS

## I. SUGAR CANE TRASH <sup>1</sup>

A. BONAZZI

*Chaparra Agricultural Experiment Station, Cuba*

### INTRODUCTION

Like all accumulations of vegetable substances on the soil, cane trash becomes of importance to the soil biologist by virtue of the modifications it may effect in the soil biota, and the changes it undergoes itself by the action of this biota.

Sugar cane trash is economically one of the most important plant residues in the tropics. It accumulates on the soil throughout the year in the form of a covering of dead leaves and leaf sheaths, falling from the acropetally growing cane and, if not disturbed or destroyed by fire, remains there after cutting, protecting the underlying soil from undue evaporation. The green tops, which at crop time are removed from canes that go to the mill, are seldom allowed to accumulate in the field in abundance, since they constitute one of the chief sources of fresh fodder for the work animals of the plantation.

It is, therefore, necessary to emphasize that cane "trash" in the restricted meaning of the word, as used in Cuba, is made up chiefly of materials which fall from the mother cane in a dead or nearly dead condition. In fact, the leaves and leaf sheaths of the lower internodes of a cane are, with very few exceptions, absolutely dry and brittle, even though still attached, by the time the cane has reached the height of 1 to 1½ meters.

In this feature, cane trash differs from the majority of green manures and mulch crops except straw, and it is for this reason that its utilization represents a distinctly different problem from the utilization of the average organic manure. It is made up chiefly of skeletal tissues with carbohydrates as its main chemical constituents.

In warm countries, where processes of oxidation generally take place at a rapid rate, the rapid disappearance of cane trash from the soil should be expected. On the contrary, however, the experimental evidence presented below leads to the conclusion that under the semi-arid conditions prevalent in the eastern provinces of Cuba this decomposition takes place at a very slow pace. In fact, few of the organisms isolated from the soil were found capable of actively attacking the skeletal tissues that go into the makeup of trash, and, of these, the most active are to be found among the fungi.

Isolations were made in these investigations with the specific aim of separating from the soil mass only such organisms as are active therein

<sup>1</sup> The accompanying is only a summary of investigations which will be reported upon more fully elsewhere.

when the soil is at rest, or, to use Winogradsky's terminology, the "autochthonous" flora.

### INVESTIGATION MADE BY DIRECT MICROSCOPIC EXAMINATION

In order to gain an insight into the biological composition of this flora, an extended examination was made by direct microscopic examination, and isolation by the soil agar plate method mentioned below, of the soil from a number of plots submitted to different régimes of trash management. It was soon found that in the heavy clay soil under study the "resting or trophic" flora is made up chiefly of a few species of cocci and of very small, weakly staining, rod-shaped gram-negative, non-spore bearing organisms with very few fungi or *Actinomyces*.

It may be stated here that these soils have been under cane for many years without a rational field management and that it is only three years since the plow and cultivator have done their work therein. It is, undoubtedly, for this reason, and on account of the frequent fires to which the fields have been submitted during their past history, with consequent depletion of their organic matter supply, that the "autochthonous" flora of these soils is so limited.

Attempts to modify this flora by means of trash additions proved that whatever changes take place in the soil are ephemeral, the picture remaining characteristic only so long as there is energy material still at the disposal of the organisms under examination. This may be seen clearly in the accompanying experiments where 14 jars each containing 100 g. of the heavy San Manuel clay (2) received the following additions, incubation taking place at 30° C.:

- 1.—40 cc. water
2.       Do
- 3.—0.2 g. cane trash powder
4.       Do
5.       Do
- 6.—1.0 g.       Do
7.       Do
8.       Do
- 9.—40 cc. water soluble, alcohol soluble cane trash  
          extract, containing 0.408 g. dry extract
10.       Do
11.       Do
- 12.—40 cc. water-soluble, alcohol-insoluble cane trash  
          extract
13.       Do
14.       Do

One day after preparation and daily thereafter specimens were obtained from these soils for direct microscopic examination with erythrosine staining (7).

The soils to which only water was added had a very meagre flora, made up chiefly of small, weakly staining, gram-negative rods and very few small, oval, spore bearing cells. An occasional chain of 2 to 3 elongated cells was to be seen. As a whole, the type of vegetation in these samples was very uniform and simple. No round cells whatsoever were seen. With addition of cane trash, the type of cells to be found did not change, but their numbers increased considerably. It was only when the water-soluble, alcohol-soluble fraction of trash extract was added to the soil that a profound change in soil flora took place for a short time. An enormous increase in the variety of forms and in total numbers was witnessed, as is clearly brought out by Fig. Nos. 1 and 2. Organisms of the type of *B. amylobacter* become very numerous and active. This increase, however, is ephemeral, since an examination made of the same soil only 48 hours after addition of the trash extract (24 hours after the time represented by fig. 2) shows a tendency to return to a normal flora. Three days after the addition, the flora is similar to that found in the natural soil. Addition of the water-soluble, alcohol-insoluble fraction of trash extract does not bring about appreciable noticeable increase of soil organisms.

#### ISOLATION OF ORGANISMS BY SOIL AGAR PLATE METHOD

Organisms isolated from field soils by smearing small particles of these on solidified agar, containing soil as the only nutritive substance, were purified by repeated smearing on successive plates of solidified agar of the same composition as that given above and plating in trash-extract agar, and then maintained in our collection on trash extract agar. Some of these, which by virtue of the color changes they induced in the medium appeared to be active in the attack of the soluble portion of the trash, were used in the experiments reported below.

Samples of equal weight of freshly collected cane trash powder suspended in shallow layers of a solution of the composition given below were inoculated and incubated at 27 to 30° C. for 39 days:

Dipotassium phosphate	1 g.
Sodium chloride	2 g.
Ferrous sulfate	0.02 g.
Tap water	1000.00 g.

The same technique was followed in a study of the action of a number of fungi isolated from the soil by the same methods. In the latter cases, however, the potassium phosphate was replaced by ammonium phosphate

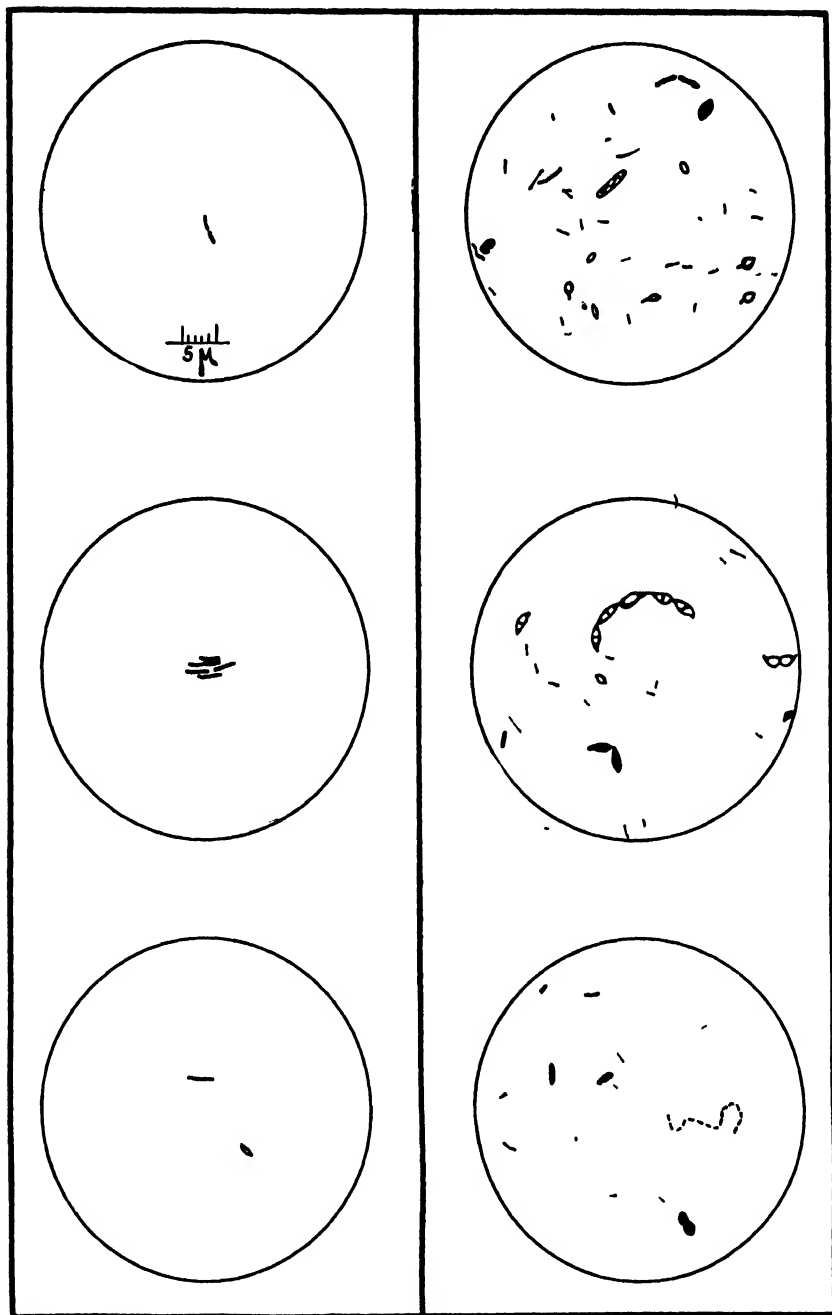


FIGURE 1.—Bacteria in soil untreated (left)

FIGURE 2.—Bacteria in soil treated with water-soluble, alcohol-soluble extract of trash (right)

so as to furnish the fungi with a source of nitrogen for their development. Incubation lasted 67 days in this case, at the end of which period the solutions were separated from the trash by filtration on tared filters, and the weight of the trash residue determined by drying the filters and content to constant weight at 100° C.

An aliquot of the dry residue was then used for a study of the substances hydrolyzable by a 3 per cent solution of sulfuric acid when this is allowed to act at 100° C. for 30 minutes. The hydrolyzate, immediately neutralized with sodium hydroxide at the end of the specified time, was separated from the residue by filtration, made to volume in a volumetric flask and used in a determination of the reducing sugars thus produced. The results of this investigation are reported in Table 2, where the reducing sugars are expressed in terms of cubic centimeters of 0.04 *N* KMnO<sub>4</sub> necessary to oxidize the Bertrand solution reduced by the cuprous oxid formed in a given volume of Fehling's liquid. The centrifugal method of sugar determination elaborated by Schaffer (6)

TABLE 1.—Organisms capable of decomposing cane trash

No.	Name of organism	Loss of dry matter mg.	Residual hydrolyzable substances	Total nitrogen in culture
45.1	Bacteria 43.44	33.8	34	
45.2	43.67	34.0	44	
45.3	43.71	56.5	40	
45.4	43.63	58.4	32	
45.5	43.69	50.7	40	
45.6	43.41	60.1	36	
45.7	43.51	36.9	40	
45.8	43.51	55.5	44	
45.9	43.42	47.8	50	
45.10	43.42	74.0	48	
45.11	43.54	52.5	48	
45.12	43.54	74.7	44	
45.13	43.25	41.5	50	
45.14	43.25	35.4	56	
45.15	Uninoculated controls	0.0	60.7	
54-2	<i>Oedocephalum</i> sp.	0.0	72	10.7
3	<i>Spicaria</i> sp.	132.8	62	11.4
4	<i>Actinomyces</i> sp.	68.2	68	14.9
5	<i>Aspergillus</i> sp.	48.8	78	14.0
6	<i>Actinomyces</i> sp.	117.1	60	16.0
7	<i>Monilia</i> sp.	69.7	64	15.0
8	<i>Monilia</i> sp.	71.7	64	15.0
9	<i>Monilia</i> sp.	0.0	76	13.4
10	<i>Monilia</i> sp.	62.9	76	12.2
12	?	110.7	64	16.8
11	Uninoculated controls	0.0	78	13.7

was used throughout these studies. Triplicate uninoculated controls were used as a basis of comparison in all cases.

It is evident, therefore, that few are the organisms capable of bringing about profound changes in the components of cane trash, even though they be of the group which should be considered truly "edaphic," and that the most easily attacked portion of the trash is that which, being soluble in water, is not precipitated on addition of alcohol.

The nitrogen content of this fraction, compared to the fraction which is precipitated by alcohol, is given in Table 2, together with some analyses made on trash obtained from the field at various stages of decomposition.

TABLE 2.—Organic nitrogen, reducing substances and cellulose of cane leaves and trash and extracted fractions of these

Description of material	Organic nitrogen	Reducing substances 0.04 N KMnO <sub>4</sub> in 1000 mg. trash	Cellulose
	per cent	cc.	per cent
Dead leaves and sheaths attached to cane	0.336	164.9	
Dead leaves and sheaths just fallen from cane	0.416	148.2	6.54
Trash on soil: only partly decomposed	0.668	85.7	7.28
Trash on soil: in more advanced decomposition	0.965	88.9	6.99
Water-soluble, alcohol-insoluble fraction	1.47		
Water-soluble, alcohol-soluble fraction	1.66		

Cellulose was determined by dissolving in ammoniacal copper oxid, filtration over asbestos, precipitation in the filtrate with alcohol, collection over tared asbestos filters, drying to constant weight and ignition. The difference between the weight of the crucibles after drying and after ignition gave the cellulose values.

Soil organisms are thus found to attack the trash and utilize immediately that portion of nitrogen which is to be found in solution; it is only after this has taken place that a further attack of the insoluble tissues begins.

During this further breakdown, the complex carbohydrates are hydrolyzed with the formation of easily fermented sugars, which then are further utilized by the soil biota in their processes of development. Synchronous with this form of attack is evinced an enrichment of the trash in nitrogen. The increase in nitrogen of fallen leaves of forest trees was found by Suchting, Wiesner and Montemartini to be due to a biological phenomenon. That this also is a bacterial phenomenon of utilization of the carbohydrates, resulting from the first hydrolytic stage, is shown by the following experiments.

## ACTION OF BACTERIA UPON FRESH AND TREATED CANE TRASH

Several portions of cane trash, obtained from the field while still attached to the mother cane, were sterilized in the autoclave and brought to optimum moisture conditions with a solution made up as follows:

Dipotassium phosphate	1 g.
Magnesium sulfate	0.4 g.
Ferric chloride	Traces
Tap water	500 cc.

To each flask were also added 500 mg. of precipitated calcium carbonate. Some flasks were left uninoculated to serve as controls, some were inoculated with *Azobacter Chroococcum Beij.*; others were inoculated with *Azobacter* after previous addition of 5 cc. of a sterile soil suspension, while others, again, were inoculated with *Azobacter* after the addition of 5 cc. of an unsterilized soil suspension.

The results of total nitrogen determination made after 29 days of inoculation are given in Table 3.

TABLE 3.—Total nitrogen determinations of several portions of inoculated cane trash

Nos.	Treatment	Inoculum	Nitrogen found	Increase in nitrogen
			mg.	mg.
1-2	Trash alone	Controls	10.74	
3-4-5	Do	<i>Azobacter</i>	10.26	-0.48
6-7	Trash and sterile soil suspension	Controls	10.74	
8-9-10	Do	<i>Azobacter</i>	10.42	-0.32
11-12	Trash and living soil suspension	Controls	13.13	
13-14-15	Do	<i>Azobacter</i>	16.36	+3.23

Evidently, even though freshly collected trash is not subject to the direct attack of *Azobacter*, it does become a favorable medium for the development and activity of this organism when transformed by the natural soil flora. This is important, insofar as trash, a pre-eminently inert and nitrogen-poor medium at the beginning of the process of decomposition, presents optimum conditions for the nitrogen fixing capacity of this type of organism (4) through the preparation activity of the trophic soil flora. Recently Abbot found nitrogen-fixation greater in sugar cane soils than in cotton soils, with a correspondingly greater abundance of *Azobacter* organisms (1).



## NITRATE ACCUMULATION IN SOILS TREATED WITH CANE TRASH

The water-insoluble nitrogen found in the trash, however, is not in such quantity and such form as to be easily utilized by that group of organisms

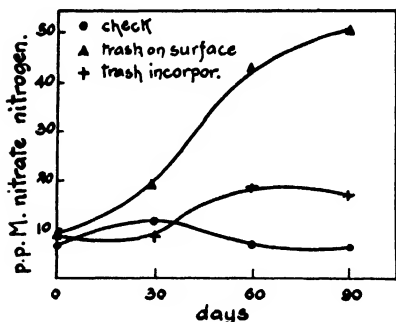


FIGURE 3

which bring about the slow, profound changes in the carbohydrate complexes of which it is composed. Therefore, it should be expected that the nitrogen balances of soils, to which trash is added, are deeply modified by this addition. In fact, when followed in the field, the nitrate accumulation from the natural soil stores of nitrogen compounds follows the path shown by the values given in Table 4 and Fig. 3.

TABLE 4.—Nitrate nitrogen of soil treated with cane trash (p.p.m.)

Treatment soil	Days			
	0	30	60	90
Check	6.10	11.63	6.46	6.78
Trash on surface	8.89	18.97	43.04	50.53
Trash incorporated	8.88	8.28	18.59	17.56

Again, when trash is incorporated in the soil in presence of nitrogenous compounds, the soil flora behaves in a similar manner, as is evinced by the curves presented in Fig. 4 and Table 5, where the quantity of nitrate nitrogen (expressed as milligrams per kilo of dry soil) found in the soil after various periods of incubation is pictured. For this experiment, samples of soil of 500 g. each were placed in jars, brought to optimum moisture content by the addition of 200 cc. of distilled water and treated as follows: where nitrate was used 0.5 g. of pure sodium nitrate salt were added, whereas where tankage was used 1 g. of this material

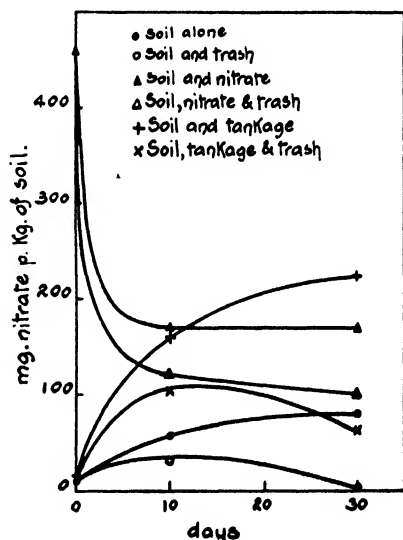


FIGURE 4.—Action of soil flora upon cane trash with various forms of nitrogen

was added. Cane trash was added at the rate of 10.75 g. of cut, dry cane leaf to each jar where needed.

TABLE 5.—Accumulation of nitrate nitrogen in soil treated with cane trash and various forms of nitrogen

	mg. nitrate nitrogen found per kg. of soil		
	At start	After 10 days	After 29 days
Soil alone	9.42	58.20	83.22
Soil and trash	9.42	30.20	5.02
Soil and nitrate	463.50	170.30	176.05
Soil, nitrate and trash	463.50	126.70	101.00
Soil and tankage	18.95	167.30	228.92
Soil, tankage and trash	18.95	105.80	68.15

From the foregoing evidence, the conclusion seems warranted that that group of organisms, normally living in a soil to which no addition has been made, the trophic flora, and which are the agents of the slow breakdown of trash, is also concerned with the utilization of the nitrates during the utilization of the carbohydrate stores of the trash.

#### RELATION OF MOISTURE AND CANE TRASH TREATMENT OF SOIL

Soil biological phenomena are enhanced in semi-arid regions by the presence of trash, on the surface or when incorporated in the soil, insofar as these additions tend to maintain more favorable moisture conditions in the soil as a medium. The diagrams given in Fig. 5 unquestionably

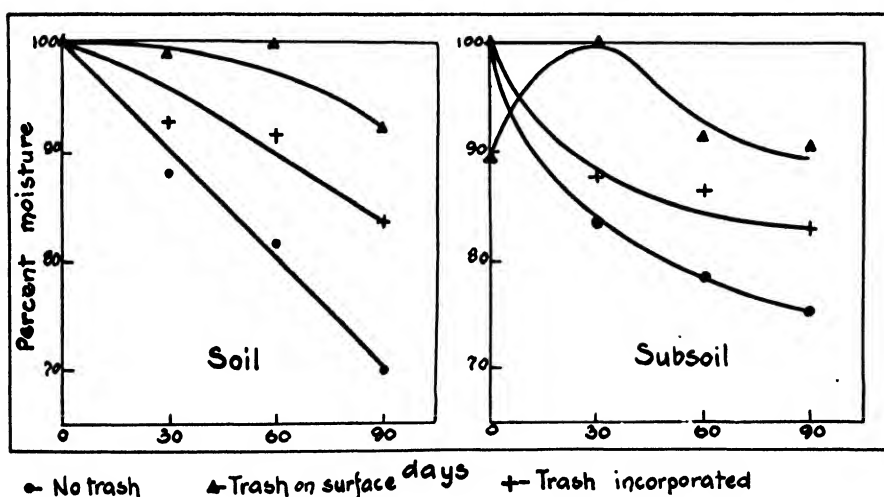


FIGURE 5.—Effect of surface and incorporated application of cane trash upon soil moisture

show the bare soil to have a greater paucity of water throughout the period of observation (6).

Interesting in this connection is the fact that the evidence indicates that the succession of activity in a soil to which trash is added is such as to tend, under natural conditions, toward an enrichment in nitrogen of the trash-soil complex by the path hereby summarized: (a) attack of the complex carbohydrates and nitrates by the autochthonous flora, leading to their transformation to more assimilable and fermentable forms, with a depletion of the stores of easily available nitrogen, and (b) a subsequent activity of the nitrogen fixing forms forced by this preliminary stage to an oligonitrophylic phase of activity while utilizing the stores of easily fermentable carbohydrates formed above. This succession of steps leads us thus to better understand the actual biology of that group of organisms which, living in a nitrogen-poor medium, such as soil, make use of the large stores of fermentable materials that find their way into the soil mass.

It may be added in this connection that solution cultures of sugar cane, where the media were renewed daily, showed that plants growing in absence of trash extract grew in a healthy state, whereas those growing in a solution of the same composition as that which supported the plants mentioned above, but to which a small daily addition of freshly prepared water extract of trash was made, soon showed signs of suffering, eventually dying altogether.

In a cane field covered by trash, then, the plant is not only contending with the soil forms for its nitrogen supply, but has, at the same time, its roots plunged in a medium that, directly or indirectly through incipient decomposition, possesses toxic properties for the plant itself.

From a microbiological standpoint then, the successive stages in trash transformation by members of the "autochthonous" microbiota, found living in the soil *in situ*, is of special interest, as well as the detoxification of the trash itself. It was found during the course of the present studies that, out of 115 isolations made from the soil of organisms of this group, by the methods outlined above, the majority were gram-negative, non-spore bearing bacteria. The few exceptions found beside the fungi could be referred to the types represented by *B. subtilis* and *B. megatherium*, which latter organism Conn (5) considered of very secondary importance in soils.

#### LITERATURE CITED

- (1) Abbott, E. V. 1926. A study of microbiological activities in some Louisiana soils. Louisiana State University Exp. Sta. Bul. 194.
- (2) Bonazzi, A. Reconocimiento Agro-Geologico de las fincas de Chaparra y Delicias Provincia de Oriente, Cuba. In press as Boletin de Minas. Direccion de Montes y Minas Rep. Cuba.

- (3) ———. 1925. Sugar cane trash as manure. *Planter and Sugar Manufacturer*, 75: 410.
- (4) ———. 1924. The mineralization of atmospheric nitrogen by biological means. IV. Internatl. Soil Sci. Conference, Rome. 111 B: 74.
- (5) Conn, H. J. 1917. Soil flora studies—iii Spore forming bacteria in the soil. New York (Geneva) Agr. Exp. Sta. Techn. Bul. 58.
- (6) Shaffer, P. A. 1914. On the determination of sugar in blood. *Jour. Biol. Chem.* 19: 285.
- (7) Winogradsky, S. 1924 Sur la méthode de la microbiologie du sol. IV Internatl. Soil Sci. Conference, Rome 1: 1248.
- (8) ———. 1924. Sur la microflore autochtone de la terre arable. *Compt. Rend. Acad. Sci. [Paris]* 178: 1236.

# RELACIÓN ENTRE LAS LEVADURAS Y LA POPULACIÓN MICROORGANICA DEL SUELO

R. CIFERRI

*Estación Nacional Agronómica, Republica Dominicana*

## INTRODUCCIÓN

Si bastantes numerosos son los estudios de las levaduras en los suelos, en relación a su circulación, y entonces a su importancia en la economía de la naturaleza como agentes de fermentaciones, y con fines sistemáticos, menos en detalle es conocida la influencia que pueda tener sobre las variaciones cualitativas y cuantitativas de los microorganismos de los suelos.

En orden cronológico, el primero a ocuparse de las levaduras en los suelos fué el Hansen (10-16), que también el primero a contribuir al conocimiento del ciclo de estos organismos en la naturaleza. Estos muy interesantes estudios fueron proseguídos ó ampliados por diferentes Autores entre los cuales deben citarse Müller-Thurgau (24-25), Berlese (2), Boutroux (3-5), Klöcker (17-21), Cordier (6), Guilliermond (9), Fischer (8), Kohl (22), Ludwig (23), De Kruyff (7), Adametz (1), y otros más. Igualmente bastantes numerosos son las observaciones de la existencia de levaduras verdaderas (*Saccharomycetae*) ó pseudolevaduras (*Torulopsidaceae*) en las flores, en los frutos, en los nectarios, etc., observaciones que emigran de nuestro campo de estudio.

Muy recientemente Starkey y Henrici (26), hicieron un interesante estudio sobre la presencia de las levaduras en el suelo, á cuyo trabajo reenviamos para más detalladas noticias históricas sobre este sujeto, y del cual hemos sacado las citaciones bibliograficas.

## MÉTODOS EMPLEADOS

Las experiencias y las determinaciones fueron efectuadas sobre el suelo de la Estación Nacional Agronómica de Moca, situada en el Cibao; el tipo de suelo es únicamente negro, umífero-arcilloso, muy fértil, profundo de un mínimo de 6 pulgadas á un máximo de 60 pulgadas, cuyo subsuelo es constituido por una masa omogénea de arcilla impermeable, amarillenta. Las parcelas escojidas, hasta fin del pasado año, estuvieron cultivadas de "yerba de guinea" (*Panicum maximum* Jacq.) una graminacea forrajera; la hierba fué entonces extirpada y el suelo repetida y profundamente arado y rastrado, sin que temporáneamente fuesen cultivadas otras plantas.

A menos de indicaciones especiales, las parcelas escojidas para las determinaciones y los ensayos, fueron las expuestas en lleno sol, desprovistas de yerbas infestas, y de una profundidad media del horizonte humico de 60. cm. Dichas experiencias fueron parcialmente efectuadas antes del período de lluvia, y en parte durante el mismo.

Las muestras fueron tomadas uniformemente a 10–15 cm. de profundidad; cada una pesaba aproximadamente un kilogramo, y la muestra media 500 g. De la muestra media se tomaron cinco veces 1 g. de tierra, que fué mezclado con una cantidad de agua lluvia esterilizada, de las cuales se hicieron las diluciones necesarias; los resultados fueron referidos á la tierra secada hasta el peso constante.

Las determinaciones cuantitativas de los microorganismos se hicieron por computación de las colonias desarrolladas en agar al albumina según Brown (pH 7.6), agar al líquido de Czapek (pH 7.2) y agar al caldo de zanahoria (pH 6.6), este ultimo medio siendo muy favorable al desarrollo de las levaduras y las pseudolevaduras (*Torulopsidáceas*). Las determinaciones fueron todas repetidas tres veces, tomando tres muestras medias.

Dejando las anchas cápsulas de Petri donde se efectuó el desarrollo en termostato a 30° C., por tres dias, se obtuvieron los resultados que se expresan en Tabla 1.

TABLA 1.—*Determinaciones cuantitativas de los microorganismos del suelo de la Estación Nacional Agronómica de Moca*

Substrato cultural	Esquizomicétos	Actinomicétos	Eumicétos	Levaduras
Parcela 1—Agar al albumina Brown	4,375,000	780,000	32,000	2
Do 2— Do	3,024,000	660,000	20,000	0
Do 3— Do	3,640,000	790,000	91,000	0
Medias	3,680,000	743,000	56,000	0.6
Parcela 1—Agar al líquido de Czapek	5,565,000	950,000	23,000	1
Do 2— Do	4,826,000	820,000	52,000	0
Do 3— Do	4,431,000	810,000	87,000	1
Medias	4,946,000	860,000	54,000	0.6
Parcela 1—Agar al caldo de Zanahoria	1,171,000	550,000	41,000	4
Do 2— Do	2,233,000	500,000	68,000	3
Do 3— Do	2,450,000	480,000	87,000	5
Medias	1,951,000	510,000	65,000	4
Medias generales	3,526,000	704,000	55,000	1.6

Como se ve, el suelo negro examinado en relación á su población micróbica, no tiene nada de particular; el número de los Esquizomicétos, Actinomicétos y Eumicétos es relativamente alto, el que se explica por el alto contenido de sustancias orgánicas, y aunque el suelo sea muy intensamente arcilloso.

Al contrario, el número relativo de las levaduras es sumamente escaso; aún en el substrato más favoreciente el desarrollo de ellos alcanzó un máximum de 5 colonias por gramo de tierra, y una media de 4.

## IDENTIFICACION DE LAS ESPECIES

De las colonias desarrolladas en los cultivos, se pudieron aislar solamente tres especies, cuyo estudio morfológico, cultural y biológico, no será tratado en este trabajo. Estas tres especies serán identificadas, provisoriamente, solamente por el género, esto es: *Torulopsis* sp., *Saccharomyces* sp. A., y *Saccharomyces* sp. B; las tres se desarrollan en colonias blancas, teniendo las dos últimas especies, un tipo de colonia distinto y fácilmente reconocible.

## EXPERIENCIAS EN PARCELAS DE CAMPO

Se hicieron cultivos de las tres levaduras en anchas cápsulas de Petri, sobre agar al caldo de zanahorias, emulsionando una traza de cultivo del hongo en algunas gotas de agua de condensación, y luego sacudiendo la cápsula de manera de diseminar los hongos mismos. Los cultivos fueron dejados a la temperatura ambiente del laboratorio (20–24° C.), y a los tres días se rasgó la patina de cultivo, y se emulsionó con agua de lluvia estéril, de manera de obtener, de cada una suspensión, la cantidad de 10 litros.

Oportunos controles ejecutados por el contaglobulos Thoma-Zeiss, aseguraron una densidad, respectivamente de 6,800 células del *Torulopsis* sp., 4,500 del *Saccharomyces* sp. A., y 2,900 del *Saccharomyces* sp., B., por cada centímetro cúbico de la suspensión.

Con la cantidad indicada de suspensión se regaron un metro cuadrado de las mismas parcelas de donde se habían originariamente tomado las muestras. Una muestra por cada parcela tomada en la tarde misma de la aspersión del líquido, dió, al cultivo en placas solo agar al caldo de zanahoria, las siguientes cantidades de células de levaduras por gramo de tierra:

*Torulopsis* sp. 150

*Saccharomyces* sp. A. 110

*Saccharomyces* sp. B. 60

TABLA 2.—Colonias desarrolladas sobre agar de caldo de zanahoria durante dos meses

Epoca	<i>Torulopsis</i> sp.		<i>Saccharomyces</i> A		<i>Saccharomyces</i> B	
	No. colonias	Aumento ó disminución	No. colonias	Aumento ó disminución	No. colonias	Aumento ó disminución
Inicialmente	150		110		60	
I decada	184	+ 34	123	+13	66	+ 6
II Do	141	– 9	94	– 6	51	– 9
III Do	88	– 62	55	–55	42	–18
IV Do	60	– 90	52	–58	33	–27
V Do	21	–129	48	–62	24	–36
VI Do	15	–135	32	–78	11	–49

Sucesivamente, cada diez días, y por la duración de dos meses, se tomaron muestras medias, y se hicieron computaciones de las mismas de colonias desarrolladas sobre agar de caldo de zanahoria, obteniendo los resultados en Tabla 2.

A los 60 días de la inmisión de la suspensión de levaduras en el suelo, se hizo el cómputo del número de Esquizomicétos, Actinomicétos y Eumicétos, sobre dos de los tres substratos inicialmente empleados, obteniendo las cifras indicadas en Tabla 3.

**TABLA 3.**—*Esquizomicétos, Actinomicétos, Eumicétos desarrolladas sobre agar albumina Brown y agar al Czapek durante 60 días*

Parcelas con inmisión de	Esquizomicétos	Actinomicétos	Eumicétos
<b>Parcela 1:</b>			
<i>Torulopsis</i> sp.			
(Agar al albumina Brown)	3,730,000	862,000	39,000
(Agar al Czapek)	5,202,000	912,000	27,000
<b>Parcela 2:</b>			
<i>Saccharomyces</i> A			
(Agar al albumina Brown)	4,151,000	661,000	36,000
(Agar al Czapek)	4,567,000	799,000	51,000
<b>Parcela 3:</b>			
<i>Saccharomyces</i> B			
(Agar al albumina Brown)	3,842,000	718,000	81,000
(Agar al Czapek)	4,220,000	784,000	72,000
Media sobre agar al albumina Brown	3,907,700	747,000	52,000
Media sobre agar al Czapek	4,663,000	832,000	50,000

Las cifras obtenidas nos indican que:

(1) El agar al líquido de Czapek fué el que reveló el mayor contenido microbiano en Esquizomicétos y Actinomicétos; el agar al albumina Brown dió cifras algo inferiores, y el agar al caldo de zanahoria cifras más inferiores todavía. Al contrario, este último substrato fué el que reveló la existencia del mayor número de levaduras.

(2) Inmitiendo en las parcelas suspensiones aguosas de las tres levaduras aisladas, el número de ellas por gramo de tierra seca aumenta en la primera década, y disminuye en las sucesivas, pero el aumento y la disminución no están relacionados entre ellos. Por el *Torulopsis* sp. el aumento inicial es del 23% y el 10%. La disminución alcanza al máximo a los 30 días de la inmisión, y sigue progresivamente, pero en cifras poco comparables entre ellas. A los 60 días de la inmisión, la cantidad de *Torulopsis* sp., *Saccharomyces* sp. A. y *Saccharomyces* sp. B. presentes en los suelos, bajó respectivamente, del 90, 71 y 81% de su cantidad inicial. El gráfico de la Fig. 2 demuestra claramente este hecho, por cuya interpretación se efectuó una sucesiva experiencia.

(3) El número de los Esquizomicétos, Actinomicétos y Eumicétos, no ha tenido variaciones notables y uniformes después de la siembra de levaduras, demostrando así que no existe una influencia de éstas sobre el restante de la población microorgánica del suelo (Fig. 1).



Acertada la gradual disminución de los números de levaduras hechadas en el suelo, me pregunté si la pérdida era debida al dilavamiento del suelo por la lluvia, ó en vez á la muerte de las células, ó á ambas las causas.

Fué establecida una nueva experiencia empleando el suelo y la suspensión de levaduras como en las experiencias precedentes, pero hechando

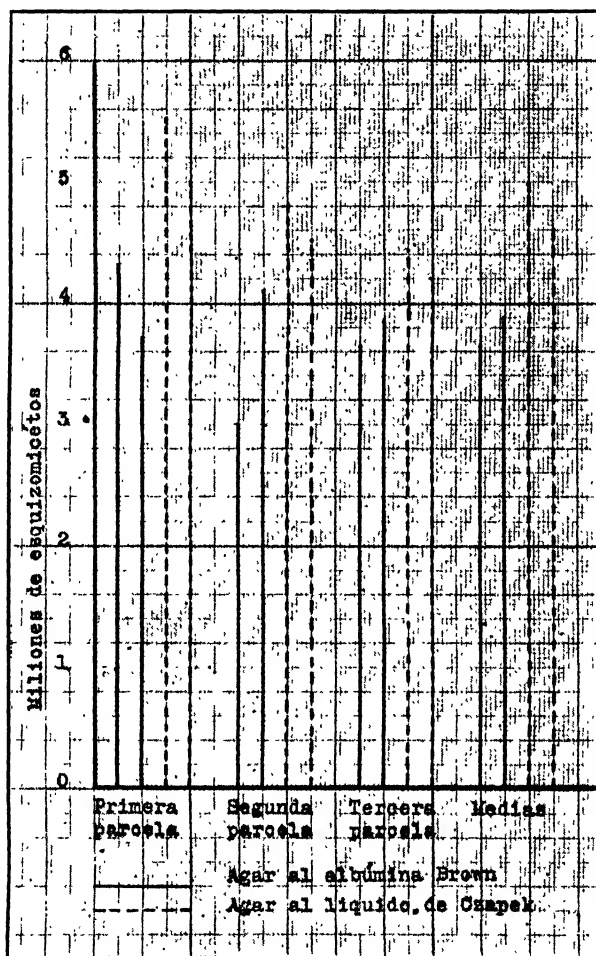


FIGURE 1.—Cantidad de Esquizomicétos desarrollados sobre dos substratos, ante (líneas I y III) y después (líneas II y IV) la vertidura de las levaduras

la tierra en una campana de vidrio de cerca 60 centímetros de alto y 25 de diámetro, y á una extremidad tubulada y coligada, por medio de un tapón y de un tubito de vidrio, á un frasquito.

Esta campana fué llenada de tierra y en élla se hechó un cultivo de cada una de las tres levaduras, de manera que, al exámen cultural, la

densidad de las levaduras mismas era de 142 células por gramo de suelo. Este aparato fué situado al aire libre, en lugar sombreado, y situado en posición tal de poder recojer el agua meteórica filtrante através de la tierra.

Fué examinado por seis veces después de caída la lluvia, el agar contenido en el frasco, en los meses de Marzo y Abril, y una muestra fué cultivada sobre agar al caldo de zanahoria. Contemporáneamente se hicieron determinaciones del número de levaduras del suelo contenido en la campana; se obtuvieron las cifras indicadas en Tabla 4.

TABLA 4.—Desarrollada de levaduras en el suelo y en la agua meteorica filtrada através de la tierra

Día de siembra	Suelo por g.			Agua de filtración por cc.		
	Número total de las levaduras	No. de <i>Saccharomyces</i> sp.	No. de <i>Torulopsis</i> sp.	No. total de las levaduras	No. de <i>Saccharomyces</i> sp.	No. de <i>Torulopsis</i> sp.
0	142	96	46			
8	131	97	34	12	7	5
15	105	81	24	2	2	
27	89	73	16	15	9	6
36	64	55	11	7	7	
39	26	21	5	9	8	1
49	22	15	2	8	8	

Como se vé, una muy relativamente pequeña cantidad de células de levaduras está capacitada a filtrar através 6 centímetros de suelo, juntamente con la agua de filtración; la cantidad de levaduras así eliminadas por filtración no está de ninguna manera relacionada con la disminución de las levaduras sembradas en el suelo, que se efectua mucho más rápidamente.

Una porción del agua filtrada fué centrifugada y el depósito examinado al microscopio; numerosos exámenes, con ó sin coloración, acertó de la presencia de las levaduras muy amenudo degeneradas ó en formas involutivas, ó esporificadas. Examinada las muestras en campo obscuro al 8° día sobre cien células contenidas en el agua de filtración, 76 estaban esporificadas y 24 nó, y al 27° día 85 tenían esporos y 15 nó. Al 8° día, cada centímetro cúbico de agua de filtración, á la numeración directa al microscopio por medio de un ocular con micrometro a red, contenía 23 células de levaduras, y al 27° día 27.

Estas determinaciones nos indican que:

(1) Las levaduras susceptibles de esporificación (*Saccharomycetes* verdaderos) tienen en el suelo una vitalidad relativamente mayor que las esporigenas (*Torulopsidáceas*); después de 49 días, la proporción de *Saccharomyces* sp. presentes se ha reducido al 16%, mientras que la del *Torulopsis* sp. bajó al 4%, y computando el número de las células

filtrada através del suelo, respectivamente el número de los dos grupos de levaduras, se redujo al 24% y al 4%. Esta confirma las observaciones de Hansen y de otros Autores, como las confirma el hecho que las mayorías de los *Saccharomyces* sp. están esporificadas (el 76% al 8° día y el 85% al 27° días en la agua de filtración).

(2) Consiguientemente, las formas esporigenas tienen una menor resistencia a las adversas condiciones ofrecidas por el suelo. Esto confirma haber el De Kruffy (7) en-

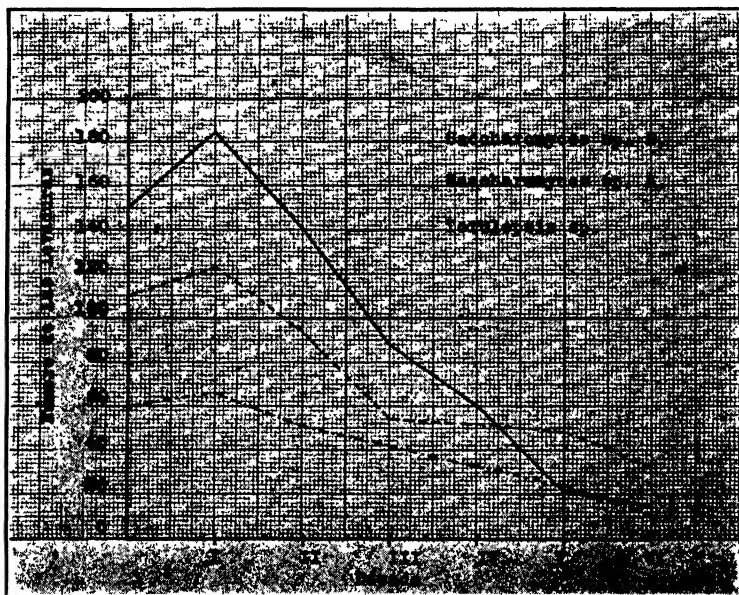


FIGURE 2.—Variaciones numéricas de las levaduras durante seis décadas

contrado, respectivamente, una pequeña proporción de *Torulopsidáceas* relativamente al contenido total en levaduras del suelo.

(3) De las células de levadura contenidas en cada centímetro cúbico de agua de filtración, solo una parte está viviente, ó á lo menos, es susceptible de multiplicarse y formar colonias; el número de levaduras computadas directamente y el número de la que se desarrollaron formando colonias fué, en el ensayo a los 8 días, en la proporción de 1.9: 1 y a los 27 días, 1.8:1. Todavía estos resultados confirman los precedentes.

(4) La disminución del número total de levaduras es progresiva, pero no constante como puede verse del gráfico Fig. 3.—La disminución relativamente mayor parece efectuarse entre el 27° y el 39° día de la inmisión de células en el suelo.

## DISCUSIÓN DE LOS RESULTADOS

A las conclusiones puede oponerse que el numero de las células de levaduras vertidas en el suelo es muy pequeña comparativamente con el número de Esquizomicétos, Actinomicétos y Eumicétos presente; en la primera experiencia, habiéndose inmitido 320 células por cada gramo de tierra, el total de estas, incluidas las 4 ya presentes, sale á 324, el que presenta, frente a los Esquizomicétos el 1: 10,882, á los Actinomicétos el 1: 169. Pero, si es verdad que el número de las levaduras hechas en el suelo es todavía muy pequeño de frente a los de los otros microorganismos,

es verdad tambien que las levaduras sembradas en el suelo (320) son muchas más de las que se encontraban naturalmente (4), representando las vertidas en número 80 veces mayor, y si esta cantidad 80 veces mayor no ha tenido influencia sobre el número de los otros microorganismos, es muy probable que el número de células que naturalmente se encuentran no tenga igualmente influencias. Estas deducciones pueden generalizarse, pues que el número de levaduras encontradas en el suelo por todos los experimentadores que se ocuparon de este asunto, es siempre muy pequeño.

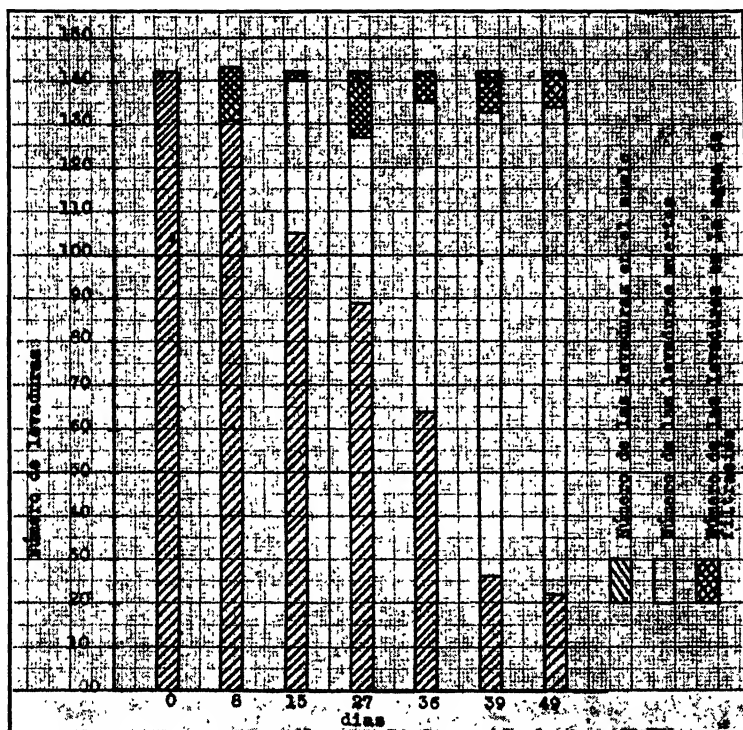


FIGURE 3.—Relación entre el número de levaduras en el suelo, en la agua de filtración, y el número de las destruidas

Refiriéndonos solamente al estudio de Starkey y Henrici (26), que más profundamente que otros examinaron esta cuestión, se vé que sobre 87 suelos examinados, el 45% contenían levaduras, pero solo el 14% más que una especie de levadura, y solo tres suelos tres especies, sin que hubieran encontrado relaciones entre levaduras y estructura de suelo, reacción del suelo, plantas presentes y épocas del exámen. Las cifras indicadas por otros Autores, como Hansen, Berlese, etc., son siempre igualmente escasas.

En vista de estos resultados, fueron descuidadas todas las otras determinaciones, que serían necesitadas en caso de una influencia positiva de

las levaduras sobre la población microorgánica del suelo, y las determinaciones cuantitativas de los principios fertilizantes del suelo, antes y después de la inmisión de levaduras.

### SUMARIO

Un suelo negro, umífero-arcilloso, situado en Moca, en la República Dominicana, sin cultivo, muy fértil, contenía por cada gramo de suelo seco, 3,526,000 Esquizomicétos, 704,000 Actinomicétos, 55,000 Eumicétos (media de nueve determinaciones, tres cada una de las cuales sobre agar al albumina de Brown, agar al líquido de Czapek, y agar al caldo de zanahoria). Las levaduras contenidas eran, en media, 1.6 g. de suelo seco; el número máximo de 4 células se obtuvo en agar al caldo de zanahoria (como media de tres determinaciones).

Las especies de levaduras fueron aproximadamente identificadas por *Saccharomyces* sp. A., *Saccharomyces* sp. B., y *Torulopsis* sp.

Fueron hechas suspensiones de levaduras en agua en el suelo, de manera de obtener unidamente 320 células cada gramo de tierra, y cada década, por dos meses, fueron computadas las cantidades presentes en el suelo, encontrándose un muy leve aumento en la primera década, y una sucesiva disminución en todas las otras disminuciones, máxima para la *Torulopsidácea*, (90%) y menos para los *Saccharomycetae* (76% en media).

El número de Esquizomicétos, Actinomicétos y Eumicétos no ha tenido variaciones notables y uniformes por el aumento de las levaduras. Se estableció que la pérdida de levaduras por filtración através de 60 cm. de suelo es sensible y que parte de las levaduras, en el suelo y en el agua de filtración, se desorganizan y se destruyen. Relativamente más resistentes son las levaduras capacitadas de producir endosporas (*Saccharomycetae*), y menos las que carecen de ellas (*Torulopsidaceae*); una gran cantidad de las primeras esporifican.

En conclusion, mientras está confirmada la condicion ambiente desfavorable que en el suelo encuentran las levaduras, tanto más si carecen de estado ascospórico, está acertado que carecen de influencias sobre la mayor parte de los seres constituyentes la población microorgánica del suelo.

### LITERATURA CITADA

- (1) Adamentz. 1886. Untersuchungen über die niedern Pilze der Ackerkrume. Diss. Phil. Leipzig.
- (2) Berlese, A. 1897. Über die Verteilung der alkoholischen Fermenten in der Nature. Ueber die Transportmittel der alkoholischen Fermente. Riv. Patol. Veg., v. 5.
- (3) Boutroux, L. 1881. Sur l'habitat et la conservation des levures spontanées. Bul. Soc. Linn., Normandie (3) 5: 120.
- (4) ———. 1883. Les ferments alcooliques. Bul. Soc. Linn., Normandie (3) 7: 73.

- (5) ———. 1884. Sur la conservation des ferments alcooliques dans la nature, Ann. Sci. Nat., Bot. 17: 144.
- (6) Cordier, J. A. 1898. Contribution à la biologie des levures de vin. Compt. Rend. Acad. Sci. [Paris] 127: 628.
- (7) Dekruyff, E. 1908. Untersuchungen über auf Java einheimische Hefearten. Cent. Bakt. (II) 21: 616.
- (8) Fischer, H. 1909. Bakteriologische-chemische Untersuchungen. Landw. Jahrb. 38: 358.
- (9) Guilliermond, A., and Tanner, F. W. 1920. The Yeasts. New York.
- (10) Hansen, E. C. 1880. Ueber *Saccharomyces apiculatus*. Hedwigia 9: 75.
- (11) ———. 1881. Recherches sur la physiologie et la morphologie des ferments alcooliques. Meddel. Carlsberg Lab. 3: 159.
- (12) ———. 1882. Recherches sur les organismes qui a différentes époques de l'année se trouvent dans l'aire à Carlsberg et aux alentours et qui peuvent se développer dans la moût de bière. Compt. Rend. Lab. Carlsberg 1: 381.
- (13) ———. 1890. Nouvelles recherches sur la circulation du *Saccharomyces apiculatus* dans la Nature. Ann. Nat., Bot. 7: 185.
- (14) ———. 1895. Experimental studies on the variation of yeasts cells. Ibid. 9: 549.
- (15) ———. 1911. Nouvelles recherches sur la circulation des levures dans la nature. Compt. Rend. Lab. Carlsberg 9: 39.
- (16) ———. 1911. Sur les foyers des levures alcooliques situés de la surface du sol. Ibid. 9: 61.
- (17) Klöcker, A. 1903. Une espèce nouvelle de *Saccharomyces*, *Sacch. saturnis*. Klöcker, ayant des spores caractéristiques. Ibid. 6: 84.
- (18) ———. 1906. Die Gärungsorganismen. Stuttgart.
- (19) ———. 1905-07. Abstammung und Kreislauf der Saccharomyceten in F. Lafar. Handbuch der technischen Mykologie, Jena. 4: 184.
- (20) ———. 1909. Deux nouveaux genres de la famille des Saccharomycetes. Compt. Rend. Lab. Carlsberg. 7: 273.
- (21) ———. 1909. *Endomyces Javanensis* nov. sp. Compt. Rend. Lab. Carlsberg 7: 267.
- (22) Kohl, F. G. 1908. Die Hefepilze. Leipzig.
- (23) Ludwig, R. E. 1918. Étude de quelques levures alpines. Bul. Soc. Bot. Geneve 9: 431.
- (24) Müller-Thurgau, H. 1889. Neue Forschungen auf dem Gebiete der Weingärung und deren Bedeutung für die Praxis. Ber. Verhandl. xl. Deut. Weinbaukongress in Trier, p. 80-100. (Cf. Heinze. Cent. Bakt. (II) 11: 355. 1901).
- (25) ———. 1905. Nachweis von *Saccharomyces ellipsoideus* im Weinbergsboden. Cent. Bakt. (II) 14: 296.
- (26) Starkey, R. L., and Henrici, A. T. 1927. The occurrence of yeasts in soils. Soil Sci. 23: 33.

# THE NEMIC POPULATION OF THE SOIL

G. STEINER

*United States Department of Agriculture, U. S. A.*

## INTRODUCTION

Nemas or Nematodes occur in almost any ordinary soil. Millions of them abound in every acre of cultivated land. Soils in the Arctic or Antarctic, or on high mountain peaks, soils that are frozen most of the time, still harbor many of them. Some 800 species belonging to about 100 genera have already been described from soils and doubtless very many are still undiscovered. Not until 1865 was the first noteworthy study of soil nematodes begun, and that was by Bastian, an Englishman. A few investigators have carried on the further study and development of the subject,—notably Bütschli, de Man, Cobb, Micoletzky, Menzel and a few others. It is indeed a recent branch of science and, until recently at least, has been occupied mainly in making an inventory of forms. Intensive work on nemic faunas has been carried on chiefly in central Europe and in the United States; tropical soils have been little studied. Therefore, our present knowledge of soil nemas is, for the most part, a result of faunistic studies and of observations made while studying plant-infesting forms. Hence there is still a wide field for future work, the whole complex of ecological and physiological problems barely having been touched, and but few carefully planned investigations having been made. From either a theoretical or practical standpoint it is an extremely promising field;—the sciences of zoology, physiology, ecology, hygiene, horticulture, forestry, plant pathology, entomology, and of soil fertility, are all interested in its development.

Lest the author seem over enthusiastic, it is well to explain a few points:

What are soil nemas? As commonly used the term soil nemas embraces only the nemas found free in the soil. This definition, however, is not comprehensive enough. Many nemas, parasitic in higher and lower animals and in plants, may pass some period of their life free in the soil; and again, many typical soil organisms have their nemic parasites which belong to the life association of the soil as well as their hosts. Even parasitic nemas of man pass part of their life cycle in the soil, e.g., the hookworm and others. If we consider that molluscs, arachnids, myriapods, insects, crustaceans, and platyhelminths, yes even nemas, have their nemic parasites, the significance of nemas in soil biology is further augmented, and nemic life in the soil falls into a number of ecological groups,

among which the interrelationships are very close. The relation of some of the above-mentioned animal parasites to soil life, however, is remote and of a negligible character so far as our knowledge goes; but some are more significant, e.g., the mermithids. Free-living and plant-parasitic nemas are, however, the chief ecological groups to be considered; and it is mainly to these we give attention here under the term soil nemas.

### NUMERICAL SIGNIFICANCE OF NEMATODES

In determining the importance of nematodes in soil biology, their numerical significance should first be ascertained. Is their number in the soil so great that they deserve the attention of the soil biologist even though we consider only the so-called free-living forms? A few attempts have been made to procure a census of soil nemas. Unfortunately, all such attempts hitherto made by other than nematologists are open to severe criticism, because usually most of the soil nemas, consisting of rather small forms or of the larvae of larger ones, were left out of the calculation, and the resulting figures were, therefore, most inaccurate. Even estimates made by nematologists give only minimal figures, probably far below the truth, for such a census of the nemic population in a given soil sample is very tedious and presents many difficulties. There is no method today which would enable us to include accurately in such a census the egg stages and forms living upon, and in, roots. Furthermore, the *complete* isolation of all these nemas from the soil is technically not yet possible.

Besides these technical difficulties, there are others. First, an even distribution of soil-nemas over a wide area may be rare. Physical and chemical conditions, nature and stage of development of the vegetation, amount of dead or waste organic material, depth to which roots penetrate, presence of other soil-inhabiting animals with their feces and waste food,—all these are factors greatly influencing the distribution of soil nemas. Decaying organic material or germinating seeds are usually points of high concentration of nemic life, where millions of individuals gather. The amount and distribution of moisture in a soil greatly influence the density of the nemic populace. In general nemas prefer light soils to heavy ones; neutral soils to acid ones; although here it is well to note that each kind of soil has its specific nemic forms.

Thus it is evident that even an approximately homogeneous distribution of nemas in the soil is quite unlikely, and that the taking of a census is a very complicated matter. Cobb attempted to work out methods for covering all these conditions. His estimates must be regarded as the most nearly perfect ones so far made; yet he considered mainly the forms found free in the soil and those only to a depth of six inches, whereas we know that along roots and in the presence of food material nematodes penetrate far deeper than this. Observations by Cobb in California



proved that they were to be found even at a depth of 25 feet, 7 to 8 m. Here are some of Cobb's figures:

		Minimum number of nemas per acre, top six inches (15.2 cm.)
From Missouri corn field		648,000,000
Do North Carolina Do	.	242,400,000
Do New Jersey Do		129,600,000
Do Rhode Island Do		610,800,000
Do New Hampshire Do		99,600,000
Do Minnesota Do		121,200,000
Do Vermont Do		580,000,000
Do Kansas Do		278,400,000

To these figures of Cobb we might add an example of ours for acid forest soil. Here only the top inch (3cm.) layer of soil was considered. The estimates were made in May.

From a wood near the Potomac River below Great Falls, Va.      320,000,000

In addition there are given here estimates made by Thorne for two fields in Utah, each of one acre and planted to sugar beets which were infested with the sugar-beet nema. In these estimates one plant parasitic form was included, but not the rest.

		Top 2 feet (about 72 cm.)
Field on Marion Bullen Farm, Lewiston, Utah		12,044,111,000
Do Davis Farm, near Salem, Utah		15,914,272,000

A careful survey and census of all nemas remaining in and on the roots would naturally have raised the estimate much higher.

All the above figures are minimum ones; but they are sufficient to prove the numerical importance and significance of the soil nematodes. Such a large component of the life association of the soil cannot be without remarkable influence.

## CHIEF FUNCTIONS OF NEMATODES IN SOIL LIFE AND SOIL FERTILITY

The main significance of soil nematodes in soil life and soil fertility, however, lies in their activities and not in the amount of organic matter they represent, which is rather small compared with some other soil animals and soil plants. The average soil nema is rather small, around 1 to 1.6 mm. in length; the minimum length is around 0.3 mm. and the maximum about 18 mm.—not considering the mermithids and other parasites, which may reach 80 cm.; yet the nemic activities in the soil are extremely manifold.

Among the important relationships that a member of a life association has with its fellow members are those connected with food. Soil nemas consume organic material, relying mainly on the autotrophic plants but

also on bacteria and fungi, as well as on soil-inhabiting animals. Plants, dead or living, undoubtedly constitute the bulk of the food of soil nemas; in comparison the amount of animal food is much smaller. Some soil nemas are omniphagous, consuming almost any kind of food, living or dead, whether plant or animal; others are vegetarians, some carnivorous. Still others are more restricted in their choice of food, preying on a single animal or plant group etc.; some are highly specialized in the matter of food. In general the menu, as far as we know, comprises detritus, dead organic material of any kind in any stage of decay, algae, fungi and all other plants, protozoa, tardigrades, oligochaetes, rotifers, other nematodes and eggs, larvae and adults of insects. How far other members of the life association of the soils are involved as a source of food for nematodes cannot be stated. The main sources are, undoubtedly, the higher plants; chiefly the soil nemas are gathered about their root systems. Some enter the roots, some suck the tissue from outside, some feed on bacteria or fungi connected with the roots or live on waste products, dead or decaying parts, etc.

The so-called saprophagous nemas, in most instances, propagate rapidly so that a few specimens reaching a center of decay multiply to millions in a few days. In the humification of organic material, these saprophagous nemas play an important part. Some nematodes digest bacteria, but in other cases it appears to be the products of the bacterial growth that are digested, while the bacteria themselves pass out through the anus. Nemas have been observed in bacterial root nodules of plants. Fungi are the food of other nemas (*Plectus armatus*, *Bunonema*, etc.) It has been observed that in some instances the spores of the ingested fungi pass apparently unharmed through the intestine, only the mycelium being digested. Algae constitute the food of other nemas (*Monhystera*). Diatoms especially have been observed again and again; but other algae are certainly eaten as often,—the diatoms being observed more frequently because their undigested characteristic shells can be seen in the intestine.

Some soil nemas are predatory, feeding on tardigrades, rotifers, oligochaetes, and, to a considerable extent, on other nematodes. Up to 70 larvae of the root-knot nema have been observed killed, and partly eaten, by a single *Mononchus papillatus* in a single day. As the root-knot nema is a very serious pest, these predatory forms are useful in the control of such pests. Methods for gaining control of plant injurious forms by utilizing predatory forms are being studied and may lead later to practical results. Our meagre knowledge of the food habits of soil nemas is a handicap here. It is extremely difficult to observe these minute organisms in the act of feeding, and, because of the nature of their ingestion, it is often impossible to determine the origin of the food by examination of the intestinal contents. Undoubtedly, however, the higher plants furnish very much of the food. Although the mouth armature in soil nemas

varies greatly, thus indicating great diversity of food and of feeding habits, yet, as Micoletzky points out, nearly half the species inhabiting the soil are spear-bearing. A chief function of the nemic spear is to puncture tissues, thus preparing an opening through which the more or less liquid food material is sucked or pumped with the aid of the muscular oesophagus. Quite frequently animals may be attacked by spear-bearing forms, e.g., oligochaetes by certain dorylaimi, many insects by various triplonchs.

The high percentage of spear-bearing forms in the nemic fauna of soils points undoubtedly to this,—that the higher plants constitute a main source of food even for species which are not strictly plant parasites. Roots, cormes, bulbs, tubers, stems, leaves, flowers, seeds,—all parts of plants and even trees are attacked by these nematodes. Tufts of mosses and of other plants abound in them. In many cases these forms are parasitic; in others, this term cannot properly be applied.

Soil nemas have their parasitic enemies—bacteria, fungi and especially amoebaporida, which, it is believed, may markedly reduce the numbers of the nemic populace, especially in the late summer. To these protozoic parasites Micoletzky and Thorne have turned their attention. To what extent soil nemas become the prey of other animals is not known. Dead nemas may disappear suddenly, presumably by bacterial dissolution and action of the soil water.

Soil nemas lead a vagrant life, some being extremely sluggish, others very active. The greatest activity is shown in the larval stages. How extensive an influence the movements and locomotion of this army of little beings exert on the mechanical conditions of any given soil is still a question. A result of great importance from the activity of the soil nemas is the spread of other soil organisms; it is evident that bacteria and fungi are in many instances transported and distributed by soil nemas. Evidence is increasing that nemas thus act as a factor in the spread of some so-called virus diseases, e.g., tomato mosaic. Besides which they are known to be carriers of diseases of higher plants. *Tylenchus tritici*, a parasite of cereals, has recently been found to carry *Pseudomonas tritici* and *Dilophospora alopecuri*, a bacterial and a fungous disease of cereals. Furthermore, nemas that enter or puncture parts of plants prepare a road for the entrance of other organisms.

Observations of a very peculiar nature may perhaps indicate a strange activity of some saprophagous forms belonging to the genera *Diplogaster* and *Rhabditis*—under certain conditions these nemas swallow air regularly. The ingested air bubbles can be seen gliding down the oesophagus; as they enter the intestine, they at once vanish. The disappearance of the nitrogen contained in these air bubbles is very remarkable, yet this “eating of air” is done by some nemas systematically. A species was named *Diplogaster aerivora* by Cobb because of its air-swallowing propensities.

Nothing as yet has been ascertained concerning the metabolism of soil nemas nor as to the extent or manner in which they influence their environment by their respiration, secretion and excretion. It may, however, be stated that in cultures of saprophagous forms the medium, after a certain time, becomes toxic for the nemas, apparently by the accumulation of their waste metabolic products. But in these forms the metabolism must be of extreme intensity because of their quick growth and development. In addition, we know that certain soil nemas are extremely susceptible to lack of oxygen, but others can, it seems, stand an almost complete absence of it. Here, then, we have an almost continuous gradation to conditions as they exist in intestinal parasites, which, as in the case of *Ascaris*, do not need oxygen for their metabolism.

The question arises, how are the nemas adjusted to all these activities in the soil? Here we see again a number of those harmonies between organism and life medium which ever anew fascinate the student of nature. The snake-like body of the nema, with an almost complete absence of appendages, is extremely well fitted for life in the soil; its sensory apparatus, and many other characters specially adapt it to its environment. However simple this sensory apparatus may be, it provides the needed relations with the environment, both near and far.

The nemic fauna of the soils is a typical fauna of the shade,—of the dark. In marine nemas, eyespots or organs for the perception of light are quite frequent; freshwater forms also have been found with eyespots, but no typical soil nema has yet been found to possess them. The soil nemas are indeed real beings of the darkness. As far as can be judged today, their sensory relation with their environment is restricted to the sense of touch and to chemical perception. It seems practical that food and the other sex are located in many instances by means of an extremely finely developed and highly specialized chemical sense. It is thought that the latter is at least partially located in peculiar organs typical to nemas, placed close to the head end and called amphids, or, less happily, lateral organs. In addition, there are sensory papillae on the head which in many cases show distinct morphological differentiation, and are thought to be mainly tangoreceptors, used especially for sensory relations with the near environment ("die Nahwelt"), whereas the chemical sensory apparatus may communicate with the more distant environment ("die Fernwelt"). The tangoreceptors of the marine and freshwater nemas are long and setae-like, while those of the soil nemas, in the great majority of cases, are short and often hard to be distinguished though there are marked exceptions. There seems to be thus indicated an adaptation to life in the soil, where naturally all protuberances on the body are a hindrance.

As a mediator in the sensory relations with the more distant environment, undoubtedly the soil water plays an important rôle; although the

air in the soil doubtless also may have its significance. It has been observed that certain rhabdites and diplogaster, as well as hookworm larvae climb elevations of moist soil and swing and undulate the anterior part of the body in the surrounding air as if testing it. Air and water movements in the soil are, therefore, of the most direct importance to soil nemas. If the soil water flow, or the soil air movement, be favorable, distant food and the distant sexual partner may thus be recognized and located by "scent." How delicately and effectively this sensory apparatus works may be conceived from the fact that some plant parasitic nemas are able to recognize and distinguish various host plants in the soil at a distance of several meters; they may even distinguish varieties of the same plant. Some species of *Rhabditis*, which can be morphologically distinguished only with great difficulty, when brought together cannot be hybridized,—presumably because of this highly specific sexual sense.

Another interesting point in the relations between various media and nemas is that in the ocean and freshwater almost all nemas are bisexual; but in the soil there is an astonishing number of species which are syngonic or parthenogenetic—no males being needed for propagation.

As to the seasonal behavior of the nemic fauna of the soil, many species of nemas are to be found at any time of the year. On thawing frozen soil in winter, some forms soon begin to move and to exhibit full activity. The few investigations and observations that have been made on this subject, however, prove that there are certain obvious changes in the number as well as in the kind of species during the various seasons. Some species prevail during the cold season, others during the warm season. Under temperate conditions the maximum production of soil nematodes is in late fall when large amounts of organic material become available; the minimum production naturally occurs in January and February.

In conclusion, it is to be said that our present knowledge of the significance of the nemic population of the soil is still very restricted. But many signs indicate that the near future will see an intensification of research in this field. May soil nematology be esteemed equally with other branches of soil biology and soil science! It certainly deserves such consideration from a theoretical, as well as from a practical, point of view.

# STUDY OF HUMUS: CHERNOZEM HUMUS AS A POLYDISPERSE SYSTEM

N. B. VERNANDER AND A. N. SOKOLOVSKII<sup>1</sup>

*Institute for Soil Research, Kharkov, U. S. S. R.*

## INTRODUCTION

Investigations carried out thus far have not determined clearly the properties of humus, the soil component of such practical and theoretical importance. The work done until now was on products obtained from treating the soil with alkali and acids—strong reagents. The products resulting from such treatment do not correspond in their properties to the natural components found in the soil. This has been shown by a number of investigators and recently by Shmuck and Gortner. The natural unchanged humus has not been studied.

On the other hand, the fundamental requirements for the study of the chemical composition of a substance are its physical homogeneity; for crystalloids this is accomplished on the basis of their ability to crystallize under well defined conditions. For colloids such conditions are not applicable; the measure of its physical homogeneity is the uniform degree of dispersion; it is with this phenomenon that a series of physical properties of colloid systems are connected.

Apparently soil humus as a colloid must correspond to the same conditions; its physical and chemical properties must be closely connected with its fundamental property as a heterogeneous system. These properties must also be related to the properties of the mineral soil colloids; they may behave like clay, or, as shown by Navassar, like tannin, where the fractions of different degrees of dispersion show different chemical and physical properties. From this standpoint it may be assumed that humus, or better to say its pseudo-solution, is a heterogeneous system with an extremely changeable degree of dispersion of its dispersed phase (a mixture of particles of various sizes and its properties will confirm the relation of the properties to the degree of dispersion).

The problem undertaken was to separate the humus into fractions of various degrees of dispersion.

## EXPERIMENTAL

As a starting material for this work a sample of chernozem from the government of Tambov was used; it represents a type of deep soil (humus

<sup>1</sup> Translated from the Russian manuscript by Dr. J. S. Joffe, N. J. Agr. Expt. Sta.

horizon equals 118 cm., the percentage of humus equals 7.7 per cent). The sample was taken at a depth of 10 cm. The active clay (the fraction  $<0.001$  mm. was separated by suspending the soil material free from absorbed calcium for 24 hours in a layer of liquid 10 cm. deep) was equal to 34.24 per cent, total nitrogen 0.31 per cent, absorbed calcium, 0.94 per cent. The black active clay was obtained directly from a natural soil by forcing out the bases with a  $N$  solution of  $\text{NaCl}$ . The chlorides were removed by washing the soil with a 1.5  $N$  solution of  $\text{Na}_2\text{SO}_4$ . The absorbed calcium was removed and a dispersed solution was thus obtained. The sample of soil was mixed with a layer of water 10 cm. deep and every 24 hours the supernatant liquid was poured off and a fresh portion of water added. This operation was repeated several times. In this manner a considerable quantity of a black pseudo-solution of humus was obtained. The suspension also contained a considerable portion of the dispersed mineral fraction. The color of the solution was of an intense dark brown, reddish in diffused light, clear when in layers, 5 cm. thick.

The separation into different fractions was difficult as there is not as yet a well worked out method. It was not possible to resort to the method of settling, a long tedious process; the following method was therefore adopted:

The dark pseudo-solutions were passed through a Berkfield filter under pressure (0.4 atmospheres). At first the larger size particles passed through the filter, but gradually the filter clogged and the particles that passed through gradually decreased in size.

In the process of filtration definite quantities of the solution were taken in succession as the filtrates came through. Each sample, therefore, represented a fraction with a definite degree of dispersion and a specific speed of filtration.

The method is open to a series of objections, the main one being the fact that the fractions (except perhaps the last one) are not uniform. This process of filtration excludes successively the large particles. If, for instance, the original suspension contains all the particles  $<1 \mu$  (those that did not settle after 24 hours) then the portions which goes through with the successive filtrations will contain less and less of these particles and there will be a continuous increase in dispersion.

Another objection is the necessity of filtering continuously, as the slightest break in the filtration process causes an opening of the pores and thus an increase in the speed of filtration. Still this method gives more uniform fractions than the method of fractional precipitation by salting out.

## ANALYSIS OF HUMUS DISPERSIONS

As mentioned, the products of filtration, after definite periods, were taken for analysis. By repeating the operation twice the portions with

the same speed of filtration were combined. The speed of filtration for one liter of filtrate changes during the process as follows:

No. of samples (1 liter each)								
1	2	3	4	5	6	7	8	9
44 min.	1 hr. 54 min.	3 hr. 30 min.	4 hr. 30 min.	5 hr. 40 min.	6 hr. 43 min.	7 hr. 28 min.	8 hr. 10 min.	10 hr. 30 min.

After taking the 9th liter the filtration practically stopped; in such a manner 9 portions were obtained with a successive increase in the degree of the dispersion.

For analysis the unfiltered Fraction 0 and Fractions 1, 4 and 9 were taken. The appearance of the different fractions was different. Upon evaporation the fraction with the largest particles formed large flakes of an intensely black color; the fraction with the smaller particles gave a thin black powder with a brownish coloration.

The hygroscopicity of these fractions were as follows: Fraction 0 equalled 19.4 per cent; fraction 9 equalled 5.08 per cent. Before analyzing the fractions they were dialyzed to remove the sodium sulfate. The ash content of Fraction 0 was equal to 64.42 per cent of the following composition— $\text{SiO}_2$ , 90.6 per cent;  $\text{Fe}_2\text{O}_3$ , 1.05 per cent and  $\text{CaO}$ , 0.1 per cent. The ash content of Fraction 9 was equal to 2.21 per cent.

The dialysis of the small fraction gave perceptible quantities of organic substances to the dialyzing medium which was colored somewhat with a light brownish coloration. The large fraction did not give any such coloration upon dialysis.

The material thus obtained were analyzed by ashing, the total nitrogen determined by the Kjeldhal method. The ammonia distilled was determined both by the titration and colorimetric methods; the later gave slightly higher results.

The forms of nitrogen were determined by the Hausmann method. One-tenth to 0.2 gr. of the material were boiled for 6 hours in a flask with a reflux condenser in hydrochloric acid (1: 12); upon cooling it was filtered, washed with hot water, dried and total nitrogen determined; this gave the insoluble nitrogen in the residue. The nitrogen in the filtrate was determined by boiling with magnesia and this gave the amide nitrogen.

Unfortunately the small amount of material on hand did not permit a determination of the other forms of nitrogen. They are therefore given as calculated by difference.

The data given show a distinct difference in the composition of the



fractions. Table 1 shows that the coarser fraction is richer in nitrogen and carbon.

TABLE 1.—*Composition of the pseudo-solution humus fraction*

Name of fraction	C	H	O	N		Ash
				By titration	Colorimetric	After dialysis
0 (Not filtered through the Berkfield)	per cent	per cent	per cent	per cent	per cent	per cent
	56.55	5.75	34.38	4.25	4.27	64.42
1				4.18	4.05	
4				3.7	3.73	
9	36.66	7.2	52.64	3.5	3.62	2.21

It has been pointed out elsewhere<sup>1</sup> that humus colloids<sup>2</sup> age, and it is possible on this basis to establish a genetic relationship between the particles of various degrees of dispersion. At first the particles are highly dispersed, then in the process of aging they form coarser aggregates; with the change in physical structure chemical changes also take place. The colloids become dehydrated and enriched with carbon and nitrogen. The same phenomenon occurs in the slightly colored substances produced by the anaerobic decomposition of plant residues which under the influence of the oxidizing processes of the air become darker and attain the nature of colloids. This has been shown in the seventies by Prof. Levakovskii from Kharkov.

Table 2 shows several differences in the distribution of the forms of nitrogen in the various fractions. Fraction 9 occupies a specific place giving the highest percentage of nitrogen in the insoluble fraction and amido nitrogen; there is also a regular decrease in these two forms of nitrogen with the increase in the coarseness of the particles of the insoluble fractions. The diamino and monoamino acid nitrogen taken together give a reverse picture—namely the increase in the total of these forms with the change toward the more coarser fractions. The smallest fraction is an exception in its lower content of mono- and diamino-nitrogen.

There is a definite analogy in the change of the chemical composition of the fractions beginning with the smaller aggregates up to the larger ones when compared with the composition of the well known series: green wood, brown coal and anthracite.

Undoubtedly the changes are connected with the processes of ageing of colloids; alongside with the physical changes intramolecular changes

take place in the organic substances of the soil, the humus, which is in a coagulated state because of the absorbed calcium. These changes are related to degradation, compactness, formation of closed ring compounds, etc.

TABLE 2.—Forms of nitrogen in various fractions; per cent in relation to total nitrogen

Name of fraction	Nitrogen in the insoluble residue	Amide nitrogen		Mono-and diamino acid nitrogen		Diamino acid nitrogen	
		Nitrogen in sol.	Total nitrogen	Nitrogen in sol.	Total nitrogen	Nitrogen in sol.	Total nitrogen
0	35.01	per cent 22.9	per cent 14.9	per cent 77.1	per cent 50.1	per cent 14.7	per cent 9.6
1	41.15	22.7	13.4	77.3	45.45	13.0	7.65
4	53.08	27.4	12.8	72.6	34.12	5.7	2.68
9	68.5	76.3	24.0	23.7	7.5	10.5	3.3

Undoubtedly beside the physico-chemical factors there are also some biological reactions.

This investigation is the first attempt to solve the problem, and the study is being continued.

## CONCLUSIONS

For the study of humus as a polydispersed system it is necessary to apply the methods not only of the elementary chemical analysis but also methods in connection with the degree of dispersion; it is necessary to separate the fractions of various degrees of dispersion in order to obtain a homogeneous material for investigation.

The experiments of the authors have shown relation between the degree of dispersion and chemical composition of the humus fractions.

## PAPERS NOT SUBMITTED

The following is a list of papers presented before the various sections of Commission III but which were not submitted for publication. The page references given are those of the Abstracts of the Proceedings of the First International Congress of Soil Science, unless otherwise indicated:

### **DIE BEDEUTUNG DER BAKTERIEN FÜR DIE FRUCHTBARKEIT DES BODENS**

J. STOKLASA

*Technischen Hochschule und Staatlichen Versuchsstationen, Prag, Czechoslovakia*

Page 1

### **A STUDY OF THE PROTOZOA OF SOME AMERICAN SOILS**

H. SANDON

*Rothamsted Experimental Station, England*

Page 32

Published also in Soil Science.

### **THE INFLUENCE OF LIMING AND DRAINAGE ON THE BIOLOGICAL ACTIVITIES OF A SAVANNAH (UPLAND BOG) SOIL OF EASTERN NORTH CAROLINA**

I. V. SHUNK

*North Carolina State College  
and New Jersey Agricultural Experiment Station, U. S. A.*

Page 35

### **THE EFFECT OF PROTOZOA AND FUNGI, WHEN INOCULATED INTO PARTIALLY STERILIZED SOIL, ON CERTAIN BIOCHEMICAL TRANSFORMATIONS**

C. E. SKINNER

*University of Minnesota, U. S. A.*

Page 39

### **DES MAIS SE DEVELOPPENT NORMALEMENT EN N'UTILISANT QUE L'AZOTE FIXÉ PAR DES BACTÉRIES**

G. TRUFFAUT ET N. BEZSSONOFF

*Versailles, France*

Page 49

**THE ANAEROBIC BUTYRIC ACID FORMING BACTERIA OF SOIL**

E. B. FRED

*University of Wisconsin, U. S. A.*

Page 54

**VARIETAL RELATIONSHIPS AMONG THE LEGUME BACTERIA**

R. H. WALKER

*Iowa State College, U. S. A.*

Page 65

**SUL PROCESSO DI AMMONIZZAZIONE**

R. PEROTTI

*Reale Istituto Superiore Agrario, Pisa, Italie*

Page 75

**THE ORIGIN AND NATURE OF THE HUMIC MATTER OF THE  
SOIL, AND ITS RELATION TO THE SOIL NITROGEN**

H. J. PAGE

*Rothamsted Experimental Station, England*

Page 97

**THE NATURE OF SOIL ORGANIC MATTER**

S. A. WAKSMAN

*New Jersey Agricultural Experiment Station, U. S. A.*

Page 98

**THE DEGREE OF HUMIFICATION OF THE SOIL  
ORGANIC MATTER**

G. W. ROBINSON, J. O. JONES AND R. J. EVANS

*University College, Bangor, North Wales*

Page 99

**DECOMPOSITION DE LA CELLULOSE PAR LES  
MICROBES ANAÉROBES DES SOLS**

Y. KHOUVINE

*Pasteur Institute, Paris, France*

Page 108

**BACTERIA CONCERNED IN THE DECOMPOSITION OF  
CELLULOSE IN SOILS****R. J. DUBOS***New Jersey Agricultural Experiment Station, U. S. A.*

Page 112

**THE REDUCTION OF INORGANIC NITROGEN  
COMPOUNDS BY MICROORGANISMS****J. BLOM***Royal Veterinary and Agricultural College, Copenhagen, Denmark, and the  
New Jersey Agricultural Experiment Station, U. S. A.*

Page 114

**HABEN DIE KNÖLLCHENBAKTERIEN BEDEUTUNG FÜR  
DIE FRAGE DER KALKEMPFINDLICHKEIT  
DER GELBEN LUPINE?****P. EHRENBERG UND R. REINCKE***Universität zu Breslau, Deutschland*

Page 135

**THE EVOLUTION OF CARBON DIOXIDE IN SOIL AND ITS  
IMPORTANCE IN THE GROWTH OF HIGHER PLANTS****H. LUNDEGARDH***Stockholm-Experimentalfältet, Stockholm, Sweden*

Page 150

**BESTIMMUNG DES KALKBEDÜRFNISSES VON BÖDEN MIT DER  
AZOTODAKTERPROBE****N. NIKLAS***Weihenstephan, Deutschland***LES CHAMPIGNONS PHYTOPATHOGENES DES SOLS DE  
FORÊT****J. DUFRENOY***Corrèze, France***THE BACTERIA FIXING ATMOSPHERIC NITROGEN****B. M. GUBIN***Agricultural Experiment Station, Moscow, U. S. S. R.*

**ÜBER BIOLOGISCHE UNTERSUCHUNGSMETHODEN ZUR  
ERMITTLUNG DES NÄHSTOFFBEDÜRFNISSES DER  
BÖDEN UND DEREN IMPFFÄHIGKEIT**

N. NIKLAS

*Weihenstephan, Deutschland*

**PROTEINZERSETZUNG UND PIGMENTBILDUNG BEI  
MIKROORGANISMEN**

A. RIPPEL

*Göttingen, Deutschland*

**THE DEGREE OF HUMIFICATION OF ORGANIC MATTER IN  
THE SOIL**

A. NEMEC

*Institute of Agricultural Research, Prague, Czechoslovakia*

**REVERSIBLE OXIDATION-REDUCTION IN ORGANIC SYSTEMS  
AND ITS BIOLOGICAL SIGNIFICANCE**

W. M. CLARK

*United States Public Health Service, U. S. A.*

Now available as a Bulletin publication of this Department,  
Washington, D. C.

**THE RÔLE OF DENITRIFYING BACTERIA IN REDUCTION  
PROCESSES IN SOIL**

F. C. GERRETSON

*Agricultural Experiment Station, Gronigen, Holland*



## **COMMISSION IV**





# CONTENTS

Contents for Commission III, pages ix and x

	PAGE
Hoagland, D. R., and Martin, J. C., Recent experiments concerning the adequacy of artificial culture solutions and of soil solutions for the growth of different types of plants . . . . .	381
Farr, C. H., The effect of calcium and hydrogen ions upon root hair growth . . . . .	393
McHargue, J. S., The significance of small amounts of inorganic elements in plants . . . . .	416
Sievers, F. J., and Holtz, H. F., The significance of nitrogen in soil organic matter relationships . . . . .	423
Stevenson, W. H., and Brown, P. E., Fertility studies on some peat soils . . . . .	437
Brown, P. E., The soil type as a factor in soil fertility studies . . . . .	442
McCool, M. M., The effect of soil fertilization on the moisture content, density, heat of wetting and phosphorous content of the cell sap of plants . . . . .	449
Lipman, J. G., Blair, A. W., and Prince, A. L., The influence of manure, commercial fertilizers, and lime on the chemical composition of field soils . . . . .	454
Kreybig, von L., Ein Beitrag zur Einwirkung von Superphosphat und Rhenania-phosphat auf den Boden . . . . .	465
Blair, A. W., and Prince, A. L., Types of soil and the phosphate requirement of potatoes . . . . .	477
Duley, F. L., Easily replaceable calcium in relation to returns from liming . . . . .	484
Harner, P. M., Investigations in the management of Michigan muck soils for general crop production . . . . .	492
Veihmeyer, F. J., and Hendrickson, A. H., The relation of soil moisture to cultivation and plant growth . . . . .	498
Alway, F. J., Effect of burning the forest floor upon the productivity of Jack Pine land . . . . .	514
Neidig, R. E., and Snyder, R. S., The cause of low productivity in recently cleared coniferous timber lands . . . . .	525
Stevenson, W. H., and Brown, P. E., Fertility studies of an abnormal Iowa soil ("Push" soil) . . . . .	542
Alway, F. J., and Rost, C. O., Effect of forest fires upon the composition and productivity of the soil . . . . .	546
Millar, C. E., Changes in soils long under cultivation . . . . .	577
Alway, F. J., Detection of sulfur-deficiency of soils by means of plants . . . . .	590
Lyon, T. L., Nitrogen economy in Dunkirk silty clay loam . . . . .	619
Truog, E., How plants feed . . . . .	628
Roe, H. B., Productivity of peat soil as influenced by height of the ground water table . . . . .	637
Turrentine, J. W., The world's resources in agricultural potash . . . . .	662
Ernst, F. A., Atmospheric nitrogen fixation . . . . .	680
Kelley, W. P., and Brown, S. M., Boron as a toxic constituent of arid soils . . . . .	688
Waksman, S. A., Cellulose as a source of "Humus" in the soil . . . . .	690



# RECENT EXPERIMENTS CONCERNING THE ADEQUACY OF ARTIFICIAL CULTURE SOLUTIONS AND OF SOIL SOLUTIONS FOR THE GROWTH OF DIFFERENT TYPES OF PLANTS

D. R. HOAGLAND AND J. C. MARTIN

*University of California, U. S. A.*

## INTRODUCTION

An opportunity to benefit by varied experiences, such as the present one, comes so rarely that we cannot refrain from bringing forward for discussion a number of different questions. However, all of these questions revolve around a central problem, which deals with the chemical characteristics of solutions capable of supporting the satisfactory growth of different types of agricultural plants. We have been using various means to obtain the necessary information and while extensive data are available, the presentation of the details of the experiments would be quite impossible in a paper of this type. Only a few results will be referred to by way of illustration.

Many of the relations existing between a plant and its medium can be studied far more effectively by using water cultures instead of soil cultures, yet the ultimate objective is the understanding of the soil as a medium for plant growth. Therefore, in many cases, the experiments with artificial media must be specifically planned to answer questions which have been raised by observations on the growth of plants in soils. This means that certain phases of plant physiology and of soil chemistry must be intimately related. The success of future development in Plant Nutrition will depend very largely upon the merging of two points of view often considered to be separate and independent.

In any ordinary soil all of the essential ions, except perhaps nitrate, are always present in some concentration in the soil moisture. No matter how rapidly these ions are absorbed by the plant, it is not probable, for example, that the concentration of phosphate or of potassium is ever reduced to a zero value. In other words, the soil can always supply such elements to the plant at some appreciable level of concentration, however low it may become.

This general consideration has justified the expenditure of much effort on experiments designed to answer to some extent the question: What is

the order of magnitude of the lowest concentrations of various essential ions adequate for the growth of different plants, provided the low concentrations are maintained approximately constant? It is quite clear that this is one of the basic questions in the study of soil exhaustion, crop rotation, and fertilizer treatment.

### IONIC CONCENTRATIONS OF SOLUTIONS

Three general methods of investigation are being employed in the California laboratories, (1) method of flowing culture solutions, (2) use of large volumes of solution for each plant with frequent sampling and chemical analysis of the solutions, followed by additions of the desired element in accordance with the indications of the analysis, (3) use of culture solutions in which one element is supplied from a solid medium (such as a selected soil or an artificial zeolite) in suspension, accompanied by chemical analysis of the solutions at suitable intervals. A brief experience with any of these methods gives one an appreciation of the difficulty of solving the apparently simple problem of concentration. It is an extraordinarily time consuming experiment to maintain a reasonably accurate control of concentrations during the active growth of a plant and practically no experiments have been reported in which such control was obtained and checked by chemical analysis of the culture solution.

Before continuing the discussion of concentration as it concerns higher plants, we should like to take the time to refer to some observations made on a simpler plant, the fresh water alga *Nitella*. These plant cells are sufficiently large so that almost uncontaminated cell sap may be secured from single cells. By combining the sap from many individual cells, a sample may be obtained suitable for chemical analysis by special methods. It is, therefore, feasible to determine directly the relations between the concentrations of a substance present in both the cell sap and in the culture medium in a much more satisfactory way than is possible with more complex plants. Experiments were made with the algal cells using different concentrations of bromide in the solutions in which the cells were immersed.<sup>1</sup> After allowing a suitable time for absorption to take place, samples of sap were secured and analyzed in the manner we have just indicated. The experiments proved very definitely that the gradient of concentration between cell sap and culture solution was increased strikingly when the concentration of Bromine ions in the culture solution was decreased. The relationships can be plotted approximately in the form of a logarithmic curve and may be made to fit an adsorption formula, although at the present time probably few investigators would be willing

<sup>1</sup> Bromine ion was used because it could be estimated with sufficient accuracy, even when very small samples of sap were available. It may also be added that other studies with *Nitella* cells have proved very interesting in connection with various problems pertaining to the absorption of ions by plant cells.

to say that this proved the mechanism to be one of simple adsorption. In a less direct way similar relations can be demonstrated for other substances and for many other plants. In fact, a general principle of plant nutrition is involved, which means that we can indicate one of the methods by which plants adapt themselves to solutions of low concentration.

## CONCENTRATION OF IONS ESSENTIAL TO AGRICULTURAL PLANTS

Returning now to the consideration of agricultural plants, we must conclude that while the mechanism referred to above is highly important in explaining soil-plant relations, there must exist critical concentrations of essential ions, below which the total intake by the plant in each unit of time is inadequate. What is the general magnitude of these critical values for different ions, for different plants, and for different climatic environments? It is needless to say that the complete answer to this question is far, very far in the future. At present we can cite merely some preliminary findings.

With regard to phosphate, we found some years ago, that a concentration of approximately one part per million maintained in a culture solution would permit excellent growth of barley. Teakle (11), working with wheat and using the method of flowing culture solution, obtained a somewhat similar result and found that 0.1 p.p.m. of  $\text{PO}_4$  was too low for maximum yields. Now these values are in a general sort of agreement with observations on various soil solutions studied in California, but apparently in other soils, or with other plants, a solution may maintain a  $\text{PO}_4$  concentration much lower than 1 p.p.m. and still permit adequate crop yield. This general question will be referred to again later in the discussion.

Sulfate also apparently may be adequately supplied for plant growth from solutions of relatively low concentration. We have conducted various experiments which indicate that even plants considered to have a high sulfur requirement may make excellent growth in solutions with  $\text{SO}_4$  concentrations of the order of magnitude of 5 to 15 p.p.m. if these concentrations are maintained fairly constant.

Johnston (12) conducted experiments on tomato plants with reference to concentrations of potassium, using a flowing solution apparatus of unusual capacity. It appeared that a concentration of potassium of the order of magnitude of from 1 to 4 p.p.m. was not adequate when all other factors were highly favorable. Experiments by one of the writers indicate that wheat and alfalfa also would not make maximum growth under solution culture conditions with the potassium concentration mentioned above.<sup>1</sup> An adequate concentration, however, is probably not very high,

<sup>1</sup> It should be understood that the values referred to represent concentrations maintained in the body of the solution. It is not possible to state what the concentrations

perhaps being well under 10 p.p.m. in some instances. 'A very important point to note in passing is that no fixed critical concentration exists for any essential element, since the climatic complex is involved very definitely. Direct evidence in support of this statement is available.' Furthermore, no answer is yet forthcoming to the question of how far such values for any given ion are affected by the concentrations of other ions present. Since one ion can affect the absorption of another it is not unreasonable to suppose that critical concentrations can be altered to some extent by changing ionic relations even though in higher ranges of concentration such relations, from the point of view of crop yield, may not be important over a great variety of solutions, as we have had occasion to point out frequently.

• Certainly, it would be a great achievement if any one plant species could be exhaustively investigated with reference to the points we have just suggested, but even then it would be necessary to face the fact that the agricultural investigator must deal with plants of many types. • How and why do they differ in their relations to the soil? This question obviously has both scientific and practical importance in a high degree, but the incompleteness of our understanding of this phase of plant physiology is clearly evidenced by the lack of agreement among different investigators and by the deficiency of experimental data in support of the various theories which have been advocated. •

### ABSORPTION OF IONS BY DIFFERENT PLANTS

Concerning this problem, one of the first questions it seems desirable to study has to do with the actual absorption of ions by different plants when they have access to the same culture solution, omitting the complications of the soil. Some experiments of this type were reported from this laboratory several years ago by J. D. Newton (8) and subsequently additional work has been carried on. A few experiments only will be noted. In one of these, buckwheat and barley plants were grown together in the same containers, so that the roots of both plants at all times, had access to the same solution as nearly as possible. Under these conditions, certain very significant differences were obtained in the composition of the plants. It might be thought, perhaps, that the buckwheat plants would have contained very much higher percentages of calcium than the barley plants, but such was not the case. The outstanding difference was found in the relative percentages of potassium, barley containing a far higher percentage of this element than buckwheat. As will be shown later, a different conclusion might be arrived at on the basis of data from soil cultures.

If the percentage composition of a plant is to be the basis for differentiating types of mineral absorption and of internal metabolism the method

---

were in the films of solution surrounding the root surfaces. It remains therefore to be determined how growth would be influenced under conditions which would permit a still more rapid renewal of the solution of low concentration.

of solution culture offers many obvious advantages over that of soil culture, but even when the solution environment is controlled, the simple consideration of the gross composition of plants in terms of percentages may be misleading. It is difficult or impossible to know when we have comparable stages of growth with different types of plants and perhaps even more important, structural differentiation varies greatly. Buckwheat plants, for example, develop stem tissue in much greater proportion than most of the grasses. Chemical studies on dissected tissues of various plants show that the percentage of potassium may be higher in the stem than in the leaf and that other elements also will be distributed unequally. The question then arises, how would the interpretation of data on plant composition be modified if perfectly comparable tissues could be analyzed?

It would seem that characteristic internal differences between plants in regard to the absorption of the various ions might be evidenced more clearly by experimenting with very simple solutions over short periods of time, using as absorbing systems, actively growing plants which have reached a suitable stage of development in some favorable complete culture solution. We have made many experiments of this kind and the results are at least very interesting. To cite only one or two observations, it was found that barley differed from certain other plants, among which were buckwheat and cucumbers, in that the latter plants absorbed from calcium nitrate or calcium chloride solutions almost the same number of equivalents of anions and cations whereas barley absorbed the anions more rapidly than the cations; strikingly so in the case of nitrate. In analogous experiments with citrus seedlings, Haas and Reed (3) observed a preferential absorption of calcium. Notwithstanding the great difficulty of really proving that different plants have different inherent powers of absorption, we must conclude, on the whole, that such is the case, although it is far from clear what factors are involved.

### PHYSIOLOGICAL INTERPRETATION OF DATA ON SOIL SOLUTION

This brings us to a consideration of the physiological interpretation of data on the composition of soil solutions. What concentrations of the various ions must be present in the soil solution to assure adequate crop growth and how are these related to concentrations required in artificial culture media? Obvious difficulties arise in interpreting the results of experiments with artificial culture solutions in terms of soil conditions. Since it has been shown that relatively very low concentrations of essential elements present in a culture medium may suffice for the needs of the plant, the solution of the problem frequently depends upon the evaluation of the ability of the soil to maintain concentrations above critical points, rather than on the determination of concentrations present at a given



moment. It is clear that in comparing plants growing in an artificial culture solution with plants growing in a soil, two opposing influences affecting absorption are involved, namely, extent of actively absorbing root system and movement of solutes to root surfaces. In a flowing solution culture, this movement would keep the solution in contact with root surfaces renewed very rapidly. Such a culture condition might find its equivalent in a soil capable of rapidly renewing the soil solution from the solid phase, but even when the renewing power of the soil is limited another factor may tend to offset this limitation. Plants usually develop relatively much larger root systems in soils than in solutions, which may tend to compensate for low concentrations, provided suitable volumes of soil are available for root dispersion. Obviously critical concentrations may not be identical in soil solutions and in artificial culture solutions. However, our present indications are that in the case of certain elements the general magnitudes are sufficiently similar to lend much interest to the experiments with artificial culture solutions.

One of the principal reasons why we are so often baffled in our attempts to understand the "renewing" or "supplying" power of a soil arises from our inability to experiment with the soil or soil solutions in a microscopic way. From a physiological point of view, interest must be centered on the solution present at the zones of contact between active root membranes and soil particles or colloidal surfaces, or perhaps on the solution which forms a part of the colloidal system as a whole, in accordance with Comber's suggestion. Possibly in such a system concentrations of essential ions may be quite different from those found in the displaced soil solution, as suggested for example by the work of Parker and Tidmore (10). The hydrogen ion concentration maintained in the solutions in these highly localized zones must also be of great consequence. In brief, the old ideas with regard to the importance of the extent of actively absorbing root system, the biological production of acids, and amount of colloid in the soil are to be given renewed emphasis in any attempt to determine physiologically limiting concentrations in soil solutions.

#### 'PRACTICAL OBSERVATIONS BEARING UPON PLANT PHYSIOLOGY

We may now note a few practical observations which illustrate some of the points we have been discussing. In connection with the investigations on different plants using the methods of sand or solution culture, it was decided to carry out some intensive cropping experiments with soils. Different types of plants were grown for some time in limited amounts of soil until the soil solution was depleted as much as possible, after which the solutions were displaced from the soil by the method of Burd and Martin, and analyzed for the most important constituents. The crops also were subjected to chemical analysis. The purpose and technique of

these experiments, it may be stated in passing, were not the same as those of the well known Neubauer method.<sup>1</sup>

Let us consider the results of one experiment in which the effects of the growth of buckwheat and of barley<sup>1</sup> on the soil solution of a certain soil were compared, as well as the relative composition of the crops and the total amounts of essential elements removed from similar quantities of soil. Both types of plants brought about a great decrease in the concentrations of nitrate, calcium, magnesium, and potassium in the soil solution obtained by the displacement method. In regard to these elements no significant difference between the effects of the two plants was manifest. In both cases the decrease in concentration of sulfate ion was relatively small. The concentration of phosphate was decreased by buckwheat and slightly increased by barley. This same relation was obtained in a second independent experiment, but since phosphate concentrations were very low in any case, conclusions must be drawn with caution. As far as the soil chemical system is concerned, it might be expected that a decrease in calcium concentration at the same or lower pH values would tend to produce increased phosphate concentrations.

The data on the plants themselves indicated a marked difference in composition between buckwheat and barley, a much greater difference than was found in similar plants grown under solution culture conditions. The buckwheat grown on the soil had a much higher percentage of calcium and of magnesium than the barley grown under exactly similar conditions. Further comparison of the two plants with regard to total amounts of elements removed from equal volumes of soil showed that buckwheat withdrew much more calcium, magnesium and phosphate than barley did, as well as considerably more potassium and nitrogen. These results are given only by way of illustration; others are available for such plants as radishes, turnips, rye, alfalfa, etc. All seem to confirm the common idea that the absorbing powers characteristic of different plants must be ascribed to their different abilities to draw indirectly on mineral supplies not initially present in the soil solution. It would appear that the extent or rates of growth of the absorbing root system, its metabolic activity (including carbon dioxide production) and relative permeability for various ions, must all be involved, as well as the metabolism of the aerial portions of the plant. The fact that evidence for the importance of some of these factors may not always be obtained in experiments on soils very deficient in colloidal matter or on soils having a pH value below a point at which carbon dioxide would be most effective in changing reactions, would not argue against the soundness of the conclusion as applied to a very large number of soils. In the experiments which are cited in this discussion, the root systems of all plants apparently permeated the whole mass of soil,

<sup>1</sup> The plants were not grown to maturity but were carried through the main portion of the vegetative phase.

but it is not possible to determine directly their actual absorbing area or the carbon dioxide relations at interphase boundaries.

## RELATION BETWEEN IONS AND CHARACTERISTIC TYPES OF ABSORPTION

Time does not permit an adequate consideration of the specific relations sometimes thought to exist between certain ions as a means of explaining characteristic types of absorption from soils or from artificial solutions. At times various results may seem to point to some fairly definite and general relationship such as that between nitrogen and calcium or between calcium and phosphate, but such relationships do not seem to hold consistently, as is illustrated in the present experiments. It may be suggested that the real situation is that all types of interionic effects occur during absorption, varying with the composition and concentration of the culture solution, stage of growth of the plant, and with climatic environment. It does not seem probable that any one specific relation can be applied consistently for a wide range of plants and solution environments. Moreover, we must view a growing plant as a dynamic system, rather than as a system to be explained entirely on the basis of simple chemical equilibria.

If it be true that the soil as a medium for plant growth can be understood properly only when its power to maintain suitable concentrations of essential elements in the soil solution is studied, then it is of utmost importance to consider the character of the mineral constituents best adapted to renew such solutions. If we first turn our attention to potassium, the replaceable bases of the soil immediately come to mind. Probably all investigators of soils would agree that one of the most consistent chapters in the history of soil chemistry is concerned with the development of the principle of base exchange. This principle has clarified many and varied soil problems, and naturally its application to the questions we are now interested in, has not been neglected. Indeed, it was given attention very many years ago. Yet it is not even now possible to find any general agreement concerning the importance of replaceable potassium in plant nutrition. Hissink (4) makes the statement that it is "remarkable that the changes which take place in soils between the adsorbed and water soluble substances has been given no more place in the explanation of absorption by plants."

Gedroiz (2) mentions one experiment in which all of the replaceable potassium of a certain Russian soil was removed without interfering with the ability of that particular soil to supply sufficient potassium for plant growth. As the result of an old experiment of Hopkins, (6) it was claimed by him that a soil ordinarily may furnish the necessary potassium even after it has been extracted with strong hydrochloric acid, which would certainly remove all replaceable bases. Numerous investigators have

studied the solubility of various non-zeolitic soil minerals and their ability to yield up potassium at rates adequate for plant development. In some cases the evidence was affirmative. On the other hand, in a recent article, Hissink (4) makes the following statement, referring to certain Dutch soils: "The small amount of adsorbed potassium found in the soils examined and averaging only 0.024 per cent is of greater importance for plant nutrition than the 0.826 per cent of acid soluble potassium."

The recent results obtained by Page and Williams (9) on the soils from the Broadbalk field at Rothamsted are highly interesting in this connection. The amount of replaceable potassium present in the unfertilized cropped soils was much smaller than the amounts in the soils which had been treated with farmyard manure or with artificial fertilizer containing potassium. Also the concentration of potassium in the drainage water from the unfertilized soil was definitely lower than in the drainage water from the potassium treated soils. In these long time experiments, crop yields on plots treated with fertilizers not containing potassium, do not equal the yields from the plots to which complete artificial fertilizer or farmyard manure is applied.

Recently in California, Hibbard and one of the writers, have had occasion to investigate various diseases of fruit trees apparently caused by soil deficiencies, probably involving potassium. In one group of soils, the maximum concentrations of potassium in the displaced soil solutions were very low in comparison with many other California soils. In certain samples these concentrations (at 18 to 20 per cent soil moisture content) did not exceed 5 p.p.m. of the solution. In these same soils, replaceable potassium was present in very much smaller amount than in other California soils used for comparison. The soils having the higher content of replaceable potassium also yielded soil solutions of higher potassium concentration. It is scarcely necessary to remark that such relations must be general and not exact in character. Further study is required of the relation between replaceable potassium and the total base absorbing power of the soil. •

✓The soils which had a low content of replaceable potassium possessed an exceptional power to remove potassium from solution by base exchange, when potassium salts were added to them in amounts comparable to heavy fertilizer applications in the field. In fact it was not possible to increase the concentration of potassium in the soil solution to more than a slight extent under these circumstances. Is all of the potassium so fixed easily available for plant growth? It might be assumed that hydrogen ions produced as results of microbiological activities or of carbon dioxide excretion by plant roots, would displace the needed potassium. However, chemical studies indicate that hydrogen ion concentration capable of production by these means would have a very limited effect in soils of this type. Calcium and magnesium came into solution almost exclusively

when the pH values of the soils were reduced to 4 or 5. The question is therefore raised whether all of the replaceable potassium in a soil is of equal value physiologically. Here we should like to give a quotation from Kelley and Brown (7) although the article from which the quotation is taken deals with another phase of base replacement: "Considering the great variation in the composition of natural silicates, including the true zeolites, the probable variation in the structural arrangement within the various molecules of silicates and the numerous possibilities for chemical combinations between the several silicic acids and the different bases, it is almost certain that a varied assortment of degradation products is formed during the course of weathering. The difference in the rate of replacement might also be due in part at least to the occurrence of molecular aggregates of the replaceable compounds, submicroscopic crystals in fact, some of whose chemically combined bases occur on the interior of the particle and can be replaced only as a result of diffusion." We must also ask how an excess of calcium carbonate in the soil affects the ability of the plant to obtain potassium when the replaceable or easily soluble form of this element is present in small quantity.

The practical question is the following: If a soil contains a very small amount of replaceable potassium, must a considerable increase be brought about by the addition of potassium salts or otherwise, before replaceable potassium can contribute adequately to the soil solution? Another practical phase to be considered is the special case of fruit tree fertilization in which account must be taken of the fact that much of the root system may be fairly deep in the soil. To what extent can the potassium content of the soil solution in contact with such roots be influenced by ordinary fertilizer practices in soils with a strong fixing power? Is it not possible in such instances, as some California results indicate, that physiological effects can be obtained only when the amounts of potassium fertilizers applied are very much larger than the usual practices would approve?

Finally, how does soil reaction or the extent to which hydrogen ions are present in replaceable form, affect the amount of potassium fixed by the soil or the availability of potassium added in commercial fertilizer? We may expect no doubt that hydrogen ions will be replaced with great difficulty so that potassium added to an acid soil may not be fixed as largely as in soils in which the exchange complex is saturated primarily with calcium. In other words, in the former case, the addition of a limited amount of soluble potassium salt may cause a marked increase in the concentration of potassium in the soil solution, frequently accompanied by a marked response in plant growth. This question is of much interest in comparing the soils of humid and arid regions.

The general impression which we have gained concerning the potassium nutrition of plants is to the effect that non-zeolitic minerals certainly do assist in maintaining physiologically important concentrations of potas-

sium in soil solutions, but that in the majority of cases, the maintenance of especially favorable or optimum concentrations requires that sufficient potassium be present in the exchange complex to keep this element renewed in the soil solution at suitable levels of concentration. Of course in making any such statement it is necessary to realize that sometimes there may be found such adjustments between other types of soil minerals (in a sufficiently fine state of division) and certain types of plants as to cast doubt on the universal validity of the assumption made above. Perhaps we should generalize only by stating that a plant is concerned simply with the equilibrium concentration of potassium as determined by all types of soil minerals, every gradation of reactivity being represented, which point will be more fully discussed by Burd.

The case of phosphate presents analogous difficulties. The concentration of phosphate in the soil solution which is adequate for plant growth apparently may vary widely depending on soil and crop. We have examined soils in which a concentration of approximately 0.5 p.p.m.  $\text{PO}_4$  was totally inadequate for the growth of tomatoes. On the other hand Parker and Tidmore (10) report good yields of corn on a soil, the soil solution of which was found to contain only about 0.1 p.p.m. of phosphate. Another soil from California having less than 0.1 p.p.m. of  $\text{PO}_4$  in the soil solution is found to be completely unsuited for tomatoes or wheat, but buckwheat grows fairly well on this soil.

In any discussions of phosphate concentrations, the reaction and buffer power of the soil must be taken into consideration, especially under western soil conditions, as Breazeale and Burgess (1) and Burd and Teakle (13) have pointed out. Furthermore, a difference between soils in colloid content in itself may mean that soil solutions of identical  $\text{PO}_4$  content may have entirely different physiological values. Again it is the ability of the soil to maintain adequate concentrations of  $\text{PO}_4$  at root-soil interfaces (or in the root-soil colloidal system) and extent of root system which is important. These factors are not always adequately reflected in water extracts or displaced solutions, except where fairly extreme conditions are involved. Yet it is not certain that many difficulties of interpretation may not be removed when we have at hand really adequate data on the renewing power of different soils for phosphate, and a better understanding of the effective root systems of different plants.

## CONCLUSIONS

How do all of the various considerations presented in this paper bear on the question of chemical soil analysis? The older methods of soil analysis have seemed to be so wholly empirical, and often so misleading in their practical application as to cause a loss of confidence in any chemical methods. Yet it is obvious that all of the modern work on soil solutions is a form of chemical soil examination. It is true that extractions of soils

with strong acids or the determination of the total amount of an element present are generally not at all capable of solving problems of crop production, but a certain rationality is now found in the use of suitable dilute acids in the approximate estimation of replaceable bases of the soil. Various forms of water extracts may give some clue to the ability of the soil to renew essential elements in the soil solution. In connection with the use of fertilizers, continued study of the fixation of potassium or phosphate must be of great importance. Hydrogen ion determinations have an established place in most soil laboratories at the present time. Instances might be multiplied to show that after all, the chemical analysis of the soil, in its modern forms, is in part, the basis for any advance in our understanding of soil and plant relations.

It is hoped that this necessarily very brief and incomplete discussion may have indicated a few of the most important problems in the field of investigation implied in the title of the paper. Its main purpose is to emphasize the desirability of relating experiments in soil chemistry and in plant physiology. The difficulties of such experimentation must be freely recognized, yet great progress is possible. A more definite advance is certain to be made when there become available in different parts of the world data which are reasonably comparable, so that the general limitations in the interpretation of experiments with soil solutions and with artificial culture solutions may become clear. Such comparable data are seldom obtainable at the present time.

#### LITERATURE CITED

- (1) Breazeale, J. F., and Burgess, P. S. 1926. *Arizona Agr. Expt. Sta. Tech. Bul.* 10.
- (2) Gedroiz, K. K. 1916. *Jour. Expt. Agron. Russia* (trans. by S. A. Waksman), 17: 472.
- (3) Haas, A. R. C., and Reed, H. S. 1926. *Hilgardia*, 2: 67.
- (4) Hissink, D. J. 1922. *Internat. Mitt. Bodenk.* 12: 81.
- (5) Hoagland, D. R., Hibbard, P. L., Davis, A. R. 1926. *Jour. Gen. Physiol.* 10: 121.
- (6) Hopkins, C. G., and Aumer, J. P. 1915. *Univ. of Ill. Agr. Expt. Sta. Bul.* 182.
- (7) Kelley, W. P., and Brown, S. M. 1924. *Calif. Agr. Expt. Sta. Tech. paper* 15.
- (8) Newton, J. D. 1922. *Soil Sci.* 15: 182.
- (9) Page, H. J., and Williams, W. 1925. *Trans. Faraday Soc.* 20 Part 3, p. 1.
- (10) Parker, F. W., and Tidmore, J. W. 1926. *Soil Sci.* 21: 425.
- (11) Unpublished data.
- (12) Unpublished data.
- (13) Unpublished data.

# THE EFFECT OF CALCIUM AND HYDROGEN IONS UPON ROOT HAIR GROWTH

C. H. FARR

*Washington University, U. S. A.*

## INTRODUCTION

The observation of plants growing wild in nature contributes something to our knowledge of the growth and nutrition of plants. Observations of more practical value are those made upon cultivated crops. A still more important study may be made by growing plants in experimental plots, where a more careful record of the plant, its treatment, and behavior may be kept. But so complex and variable are the conditions even in experimental fields, that slow indeed would be our progress in the agronomic and horticultural aspects of agriculture, if these observations were not supplemented by investigations under a more controlled environment.

The first step in this direction was the growth of plants in benches, flats, or pots under glass, where the humidity, watering, and temperature can be more accurately controlled and determined. But even here the complexity of the soil, from the atmospheric, chemical, colloidal, and biological aspects, is so great as to constitute a highly confusing factor in the interpretation of the results. The next step in the refinement of the experiments was the use of sand cultures. In these the medium of the root is simplified, especially in regard to its colloidal and biological features. But nevertheless the chemical, atmospheric and aquatic relations of the root play an important and a puzzling rôle. The next advance in this field of investigation consisted in the development of the aerated solution culture. This was in the direction of still greater simplification of the root medium, in that its adsorption was reduced to a minimum, and its chemical composition was known, including the dissolved gases, the essential ions, and the absence of toxic or non-essential ions. But important as are the contributions which a study of these balanced solutions have yielded, yet there is still a complexity which can only be analyzed by further simplification of the experiments. An improvement is the use of a flowing solution, which will obviate the change in ionic content, especially of the hydrogen ion concentration controlling the acidity and alkalinity of the medium. But there are also other complications involved. The criteria for the measurement of growth, whether it be dry weight increase, height, or productivity, are so coarse, as to



yield very conflicting results with a repetition of almost identical conditions. In other words there are so many factors, which effect the development of the plant, besides the ionic content of the solution, that the results after growing the plant to maturity are likely to be quite confusing. But even if we ignore the inaccuracies of measurement and the other factors involved, there is a difficulty in analyzing the effect of the respective constituents of the balanced solution, because of the mutual effects of these ions, such as antagonism, mutual precipitation, and ionic exchange. It therefore becomes advisable to supplement the typical culture solution experiments, by others in which a greater degree of simplification is accomplished.

The next logical step is therefore to reduce the solution to that of a single salt or of a single ion, in addition to those yielded by the solvent, water. But this step entails a modification also of the criteria of growth or nutrition. The higher plants will not develop to maturity without a group of at least six or eight ions available. We must therefore abandon for these experiments the study of the growth of the plant throughout its entire life cycle, and confine our attention to a single physiological feature. In other words it will be well to simplify the subject of investigation as well as the method. This can be most satisfactorily accomplished, if we can reduce the experiment to a cellular basis, and find a simple cellular process which will go on in the presence of a single ion in water, or in the presence of this ion plus one or more other ions. In this way we can get at the specific effect of the individual ion upon a cellular process, and by adding ions ascertain their antagonistic or additive effects.

Such a study, it is found, can be readily made by the use of root hairs, which will elongate in as simple a solution as calcium hydroxide, or in any of the nutrient calcium salts. In fact this material has proven highly advantageous for this type of investigation. The process studied is cell enlargement, an exceedingly important aspect of growth. This cell enlarges in only one dimension, and consequently the criterion is mathematically precise, to a far greater extent than the measurement of plant height or even of dry weight increase, so often used in balanced culture experiments. The root hairs will grow for many hours in a flowing solution in the dark at constant temperature, so that variation of light, temperature, humidity, and chemical changes in the medium due to secretion from the plant itself, are practically eliminated. Finally, not the least of the advantages of this material is that the organ of the plant studied, namely, the root hair, is the absorbing organ of the plant, and that the single ion in which it will grow in an aqueous medium is calcium, which has already been recognized as playing an exceedingly important rôle in soil problems. It is quite likely, in fact, that in addition to the relation of calcium to soil acidity, its importance is also due to its being the one element absolutely essential for root hair production.

## EXPERIMENTAL

The apparatus and the method employed are described in full elsewhere.<sup>1</sup> We will here epitomize this description by stating that seedlings of Georgia collards with radicles about 1 cm. long, were placed on the horizontal microscope in a chamber through which a solution was flowing. This was done in the evening and readings were taken upon the rate of elongation of root hairs arising on these radicles early the next morning. Measurements were made at 10 minute intervals for a period of 3 hours. The solution was of a known hydrogen ion concentration, determined colorimetrically. It was free from carbon dioxide, and aerated with oxygen. It flowed at a constant rate in a dark room with a variation in room temperature of less than 3 degrees during the experiment. The sources of the distilled water and of the chemicals employed were kept constant.

In harmony with many other physiological studies, root hair growth is found not to occur in distilled water of a pH value of 6.9 or less. As shown by the following data, they do grow in solutions of calcium hydroxide to a pH value of 12, or perhaps even slightly higher. In the following tables increments are given in micrometer spaces per 10 minute intervals. The averages are in microns per hour. The common logarithms are given for the average of these averages.

TABLE 1.—Rate of root hair elongation in calcium hydroxide solution

pH 7.9										Average	Log.
15-14-15-20-19-19-	9-10-	9-14-15-14-12-13-13-12-13-12								78.9	
10-10-10-16-17-17-	6- 6-	6-13-14-13-10-10-10-12-13-12								62.8	
8- 7- 8-16-17-17-	4- 5- 4- 5- 5- 5-10-	9-10- 8- 8- 8								50.7	
14-15-14-15-15-15-	8- 9- 8-13-14-13-12-13-12-13-14-13									74.6	
8- 8- 8-14-15-14-23-24-23-10-10-10-12-12-12-11-12-11										73.7	
13-12-13- 6- 6- 6-19-18-19-12-11-11-13-14-13-12-11-12										66.8	
4- 5- 4- 3- 4- 3-18-17-18-	9- 9- 9-12-13-12-10-10-10									52.9	
13-13-13-10-10-10-19-20-19-13-14-13-13-14-13-10-10-10										66.2	
										Average	65.8
											1.81823
pH 8.4											
10- 9-10- 7-12- 8-10-13- 7-14- 6-13-10-11-16-10-10- 7										56.9	
9- 9- 9- 8-12- 8-13- 7- 7-13-10-13- 7-12-15-10-10- 0										53.5	
7- 8- 8- 7-14- 6-12-10- 9- 8-18-15- 9-17-20-12-10- 8										62.2	
9- 8- 9-11- 6- 7-13-13- 9- 2- 6- 2-13- 0- 5- 0- 7- 6										39.2	
8- 9- 8- 8- 2- 8-10-14- 8-10- 5-10- 0-13- 4- 0- 6- 3										39.2	
6- 6- 6-13- 2- 8-15-12- 8- 5- 5- 5-10- 0- 2- 3- 2- 2										35.5	
9-10- 9- 8- 7- 7-15-18-10- 7- 5-10-10- 0- 8- 5- 8- 9										48.2	
										Average	47.8
											1.67943

<sup>1</sup> Studies on the Growth of Root Hairs in Solutions, I. Am. Jour. Bot. 1927. 14: 446.

TABLE 1 (Continued).—Rate of root hair elongation in calcium hydroxide solution

															Average	Log.
pH 8.9																
7-	8-	11-	12-	9-	9-	9-	9-	10-	9-	11-	10-	11-	8-	7-	8-	50.7
10-	10-	10-	10-	11-	11-	9-	10-	9-	10-	10-	10-	11-	10-	9-	8-	55.0
8-	9-	8-	8-	6-	7-	9-	9-	9-	9-	9-	9-	9-	8-	9-	7-	45.4
9-	10-	9-	10-	7-	8-	12-	11-	12-	6-	6-	6-	4-	5-	4-	2-	39.2
10-	11-	10-	11-	9-	8-	10-	10-	10-	11-	11-	11-	8-	9-	8-	0-	51.9
10-	10-	10-	10-	3-	3-	8-	8-	8-	12-	13-	12-	5-	6-	5-	3-	41.4
6-	7-	6-	6-	11-	11-	4-	5-	4-	10-	10-	10-	9-	8-	9-	3-	39.5
6-	7-	6-	6-	10-	10-	2-	1-	2-	14-	15-	14-	3-	4-	3-	7-	31.8
															Average	45.2
																1.65514
pH 9.9																
11-	12-	11-	11-	17-	18-	10-	10-	10-	4-	4-	4-	10-	10-	10-	10-	61.2
12-	13-	12-	13-	14-	15-	10-	9-	10-	16-	16-	16-	16-	20-	20-	7-	75.2
12-	12-	12-	12-	15-	15-	10-	10-	10-	16-	15-	16-	16-	18-	19-	21-	82.4
12-	13-	12-	13-	15-	15-	8-	7-	8-	12-	11-	12-	12-	29-	28-	28-	90.2
12-	12-	12-	12-	16-	16-	9-	10-	10-	11-	11-	11-	11-	17-	18-	17-	90.2
11-	10-	11-	11-	14-	15-	8-	9-	8-	23-	24-	22-	24-	18-	18-	19-	88.6
12-	13-	12-	13-	16-	17-	11-	10-	11-	25-	26-	25-	26-	17-	18-	20-	94.3
10-	10-	10-	10-	17-	18-	7-	8-	7-	29-	30-	29-	29-	17-	18-	20-	95.4
															Average	85.1
																1.92993
pH 10.9																
8-	9-	8-	6-	5-	6-	4-	5-	4-	4-	3-	4-	0-	0-	0-	0-	25.5
7-	8-	7-	6-	6-	6-	5-	5-	5-	4-	5-	4-	5-	1-	1-	2-	25.5
10-	11-	10-	9-	8-	9-	9-	9-	9-	9-	10-	9-	9-	9-	9-	8-	51.0
8-	8-	8-	10-	9-	10-	9-	9-	9-	10-	10-	10-	6-	6-	8-	9-	48.8
7-	6-	7-	8-	7-	8-	7-	8-	7-	9-	8-	9-	9-	6-	6-	10-	53.9
7-	8-	7-	6-	6-	6-	7-	7-	7-	9-	9-	9-	9-	5-	5-	7-	42.6
7-	7-	7-	11-	11-	11-	9-	9-	10-	9-	10-	9-	10-	9-	9-	9-	51.0
															Average	41.0
																1.61267
pH 11.9																
0-	0-	0-	0-	1-	2-	1-	1-	1-								6.2
1-	2-	1-	1-	1-	1-	1-	1-	1-								6.2
0-	0-	0-	4-	4-	1-	1-	1-	0-								6.2
0-	0-	0-	0-	0-	0-	1-	1-	1-								2.0
															Average	5.1
																0.70757

It is apparent from these data that the graph representing the effect of the calcium ion upon rate of root hair elongation with varying concentrations of calcium hydroxide, that is with varying calcium and hydroxyl ion concentration, is bimodal, with the more alkaline maximum the greater. This bimodal graph for variation in hydrogen ion concentration of the medium is characteristic of a large number of physiological reactions. Its occurrence, that is, the existence of a median minimum in the

curve, has been interpreted by various authors as the result of mutual precipitation of constituents of the medium, or as indicative of the isoelectric point of the constituent proteins of the tissue involved. That it is not always, at least, attributable to the former, is shown by the above experiment in which the medium employed was a simple solution of calcium oxide in water. If it is attributable to the isoelectric point of the constituent proteins of the root hair, then it should remain at the same pH value for different media. The following data shows that this is not the case.

**TABLE 2.**—Rate of root hair elongation in calcium chloride, 0.008 molar

pH 3.9																		Average	Log.	
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
(42 hairs measured)																		-----		
																		Average	0	0
pH 4.9																				
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
(21 hairs measured)																		-----		
																		Average	0	0
pH 5.9																				
13	13	17	15	13	12	17	16	17	17	16	7	10	10	18	8	10	9	74.0		
9	10	15	13	10	10	14	13	13	14	13	13	10	7	3	0	0	0	47.9		
2	8	8	7	8	8	9	8	10	10	13	2	8	8	7	5	5	3	39.9		
10	11	16	13	10	9	10	13	15	12	15	3	10	10	10	2	5	3	54.7		
7	13	13	11	18	5	13	12	10	18	17	7	0	0	0	0	0	0	44.8		
13	13	10	10	17	8	8	11	10	16	22	2	6	4	10	9	7	0	48.5		
7	13	13	13	11	8	13	17	10	18	17	7	10	20	16	9	25	10	71.5		
7	12	10	13	15	7	15	15	3	22	5	10	10	3	10	3	7	2	52.6		
																		-----		
																		Average	54.2	1.73400
pH 6.9																				
5	10	10	13	12	12	11	15	10	10	7	10	16	14	13	5	10	12	60.6		
7	10	13	10	13	7	12	10	5	13	10	7	15	10	15	3	7	10	55.0		
8	13	9	10	10	8	13	11	8	12	11	8	11	10	13	3	10	12	56.0		
10	10	10	10	16	7	17	17	3	10	10	8	15	7	13	0	10	7	52.9		
12	8	12	10	15	16	17	7	23	10	20	23	14	10	20	17	18	8	84.0		
10	10	3	7	12	10	11	4	13	10	12	15	8	7	12	13	10	6	53.8		
10	13	10	17	10	6	13	9	15	13	10	14	10	8	13	12	13	14	61.2		
7	13	12	8	10	7	20	7	13	13	10	17	13	7	16	11	13	13	65.3		
12	9	13	13	13	9	15	10	10	25	8	10	5	12	3	0	0	0	45.7		
10	15	15	12	15	13	22	12	13	23	14	19	14	15	18	7	17	10	82.1		
13	13	13	14	13	10	20	14	7	20	10	22	18	12	12	13	13	12	77.1		
10	13	10	17	13	13	24	10	13	20	12	18	22	10	21	14	13	20	84.9		
13	12	2	10	13	16	9	12	13	8	5	12	15	10	10	15	8	7	59.1		
10	10	7	10	13	17	7	13	16	4	7	10	23	10	7	16	10	10	62.2		
12	12	8	11	14	20	10	16	19	2	13	16	21	13	10	13	14	13	73.7		
16	17	14	10	16	27	10	20	15	5	20	20	15	15	20	20	20	10	90.2		
																		-----		
																		Average	66.5	1.82282

TABLE 2 (Continued).—Rate of root hair elongation in calcium chloride 0.008 molar

pH 7.9	Average	Log.
13-13-12-10-18-10-12-15-13-11-13-13-10-14-10-13- 8	68.7	
16-11-17-18-20-12- 8-27-20-16-19-15-13-24-19-19-15-13	97.3	
15-12-18-12-17-13-18-19-18-18-22-15-18-22-20-17-21-15	96.4	
17-15-23-12-13-15-17-20-20-17-16-22-15-23-20-15-15-20	101.1	
13-21-16-17-20-13-17-17-26-17-20-16-19-18-17-20-23-12	100.2	
15-16-14-13-17-18-21-16-20-17-18-12-15-18-17-10-20-16	87.4	
15-17-18-17-20-10-16-19-18-17-20-18-17-15-16-14-23-13	94.2	
18-15-12-15-20-13- 7-23-20-17-20-15-10-18-17-13-23-10	88.9	
10-19- 8-10-15-12- 7-13-10-15-15-10-13	77.7	
13-16-11-18-15-15-12-13-12-10-15-10-11	83.4	
13-20-15-20-18-14-13-18-15-14-21-18-15	99.2	
8-12-13-12-11-10- 9-12-13-10-13-12- 5	65.3	
15-19-21-15-20-17-13-24-16-13-21-11-15	102.6	
16-17-20-13-17-17-16-19-15-16-17-18-15	99.2	
8-12-15-10-18-12-12-13-16-11-16- 7-13	76.1	
10-12-15-10-13-17-10-15-12-13-13-12-12	76.4	
Average	88.4	1.94645
pH 8.9		
12-22-13-17-20-13-17-15-15-16-11-13-20-10-15-15-16-11	84.3	
13-19-13-18-11-16-13-17-14-16-13-14-20-13-14-16-17-15	84.6	
5-12-13-12-10-13-10-10-12-17-13-13-14-16-10-16-19-12	70.6	
12-15-13-17-15-11-19-15-13-22-15-13-17-17-13-20-15-10	84.6	
13-13-11-13-13-17-15-10-15-17-16-15-17-15-20-15-15-13	79.9	
14-10-13-15-15-17-13-12-15-13-15-17-15-16-19-15-16-19	83.7	
14- 8-10-11-10-11-13-13-13-11-10-19-10-17-15-12-13-14	69.7	
18-13-11-13-20-15-15-13-20-14-13-25-15-16-19-18-13-21	90.8	
16-17-17-19-17-14-19-17-17- 20-10-10-20-16-11-16-17-10	88.0	
14-20-16-21-21-15-20-17-18-22-15-20-15-20-17-13-23-14	99.9	
10-20-13-14-13-13-17-10-20-16-11-13-13-17-10-15-15-10	77.7	
11-21-10-21-17-12-19-14-20-16-17- 8-15-16-17-17-16-11	87.7	
8- 5-10-10- 5-12- 3- 8- 5- 7-10- 7- 8- 5- 8- 5- 7- 8	40.8	
13-13-11-18-18-14-13-18-12- 7-10-19- 7-10- 4- 6- 7-10	65.3	
13-10-12-15-13-20-14-13-13-12-10-15-10-13- 4- 8- 8- 7	65.3	
Average	77.6	1.88986
pH 9.9		
13- 7- 8-19-13-10-13-12-21-10-15-10-12-13-12-13-17-16	70.3	
10-10-13-22-12-13-16-14-23-17-20-13-17-17-16-17-15-15	87.1	
10- 6-10-17-17- 8-13-15-13-23-17-13-16-17-17-10-20-13	79.6	
16- 7-10-17-18-11-14-15-23-15-14-13-16-14-13-15-15-17	81.8	
8- 7-13-17-17- 8-15-15-20-13-17-10-13-18-11-13-18-15	77.1	
12-10-13-18-19-10-16-14-23-17-17-16-17-20-13-20-20-13	91.1	
8- 5- 8-17-14- 8-11-17-14-13-13-12-10-13-10-15-14- 9	66.2	
8- 7-10-20-20- 8-15-17-23-20-12-18-17-25-15-13-17-16	87.4	
10-13-14-10-18- 8-14-10-13-17- 8-12-13-12-11-17-10-10	65.3	
18-10-17-15-13-12-15-15-13-17-15- 8-15-15-17-13-24- 8	80.9	
8-14-13-12-11-10-11-13-13-17- 7-10-13-10-12-10- 8	60.6	
8-12-13-10-17- 6-11-13-13-13-11-10-13-10-15-10-10-11	64.1	
13-14-13-17-16-11-13-20-15-12-11-15-14-13-16-14-15-10	78.4	
12-13-12-15-18- 7-12-15-12-13-12-15-12-15-17-13-20-15	79.9	
15-13-18-11-20-13-13-14-19-14-13-15-12-17-16-15-18-15	84.0	
Average	76.9	1.88593

TABLE 2 (Continued).—Rate of root hair elongation in calcium chloride, 0.008 molar

pH 10.9												Average	Log.
13-	5-12-	5-10-	5-	8-12-	7-	8-13-	8-14-10-	8-10-	8-	7		53.8	
10-13-	7-	8-	8-14-	7-13-	8-	8-12-	5-12-	5-10-	6-10-	4		49.8	
5-10-	9-10-	8-15-	10-15-	10-11-	10-11-	10-11-	10-11-	13-10-	10-10-			58.2	
10-	7-10-	8-	8-13-	11-13-	8-10-	8-11-	10-13-	6-10-	7-	7		52.9	
6-	7-	7-	3-	4-13-	10-10-	6-11-	8-	8-17-	10-6-	11-10-	10-	39.5	
5-	5-	8-	5-	8-	9-10-	8-	7-	6-14-	5-10-	10-5-	16-10-	42.0	
8-15-	12-	5-	5-13-	17-15-	10-	8-	7-10-	10-11-	16-	7-13-	5	60.6	
15-	6-14-	5-10-	5-10-	16-	4-15-	13-10-	12-16-	5-15-	16-	7		55.3	
12-	5-10-	8-12-	10-10-	13-17-	15-10-	11-14-	15-10-	11-13-	15-			65.9	
7-10-	10-10-	13-	4-13-	13-11-	17-13-	17-10-	15-11-	14-15-	15-			67.5	
6-10-	7-	6-	9-10-	10-15-	13-14-	11-12-	10-13-	7-16-	14-10-			60.0	
8-13-	11-	8-	8-10-	13-17-	7-18-	12-15-	13-12-	10-17-	13-13-			67.8	
11-	8-10-	7-	4-13-	10-13-	12-15-	13-10-	12-12-	13-10-	15-10-			61.6	
13-10-	14-11-	5-17-	15-15-	13-20-	10-15-	17-15-	10-20-	15-10-				76.1	
12-10-	11-10-	7-12-	5-16-	14-20-	10-10-	17-13-	13-17-	15-15-				70.6	
7-10-	10-	6-10-	11-13-	13-	7-13-	10-12-	13-12-	7-10-	13-	8		57.5	
												Average	58.7
													1.76864
pH 11.4													
5-	5-	5-10-	10-10-	11-11-	11-11-	11-11-	6-	6-	6-	6-10-	10-10-	47.3	
6-	6-	6-10-	10-10-	10-13-	13-13-	13-	7-	7-	7-	7-	1-1	47.3	
4-	4-	4-10-	10-10-	10-10-	12-12-	12-12-	12-	6-	6-	6-	8-8	47.3	
4-	4-	4-	8-	8-	8-	8-13-	13-13-	13-13-	7-	7-	7-9-	50.4	
6-	6-	6-	9-	9-	9-11-	11-11-	11-11-	5-	5-	5-	5-8-	45.1	
												Average	47.5
													1.67669
pH 11.9													
0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0	
												Average	0
													0

(11 hairs measured)

From these data it is observed that the graph for the growth of root hairs of *Brassica* in different hydrogen ion concentrations of a solution of 0.008 M  $\text{CaCl}_2$ , is monomodal, with steep sides and a nearly flat top slightly higher on the acid side of the mean of the range, that is, at approximately pH 7.9. The range does not extend on the acid side of neutrality to a pH of 4.9, whereas on the alkaline side it reaches to a lower hydrogen ion concentration than pH 11.4. Thus contrary to the usual situation, growth is greatest in an alkaline medium, and growth occurs to a marked degree over a wider range of alkaline solutions than of acid; furthermore the curve is monomodal and not bimodal. The curve does resemble that for calcium hydroxide in that the alkaline limit is the same, though the acid limit is at a lower pH value. Also the maximum rate of growth in the two solutions is almost identical. The chief differences in the two curves are in the location of the acid limit and in the absence of a depression at pH 8.9 in the calcium chloride solution, as compared with the hydroxide. The explanation of this absence of a



TABLE 3 (Continued).—Rate of root hair elongation in calcium chloride, 0.020 molar

pH 5.9		Average	Log.
18-15- 9-21-15-16-11-13-22-15-16-19-10-15-15-15- 5		80.9	
20-14-16-17-18-22-10-13-20-13-21-16-12-12-14-14-14-14		84.9	
27-13-15-18-17-20-15-15-20-13-27-20-12-12-16-16-16-17		96.0	
24-26-10-20-10-20-22-15-16- 9-10- 5- 2- 3- 0- 0- 0- 0		59.7	
24-26-17-20-20-17-18-23-12-10-10-16- 7- 7- 0- 0- 0- 0		70.7	
20-20-10-17-15-15-10-13- 7-13- 2- 2- 1- 1- 0- 0- 0- 0		42.6	
13-13-11-13-13- 7-15-12- 8-10- 8- 0- 0- 0- 0- 0- 0		38.2	
13-16-11-13-16- 7-17-20- 8-10-15- 7- 3- 7- 0- 0- 0- 0		50.7	
13-13-16-13-17- 4-16-17- 8- 9-13-13- 0- 0- 0- 0- 0- 0		44.5	
15-12-12-13-16- 9-18-15- 8-11-13-18- 2- 3- 0- 0- 0- 0		47.6	
10-10- 9-15-13- 7-15-15-13-13- 4-13- 4- 0- 3- 0- 0- 0		46.7	
13-17-17-13-17-10-23-14-10-17-10-12-22- 0-13-10- 0- 6		66.2	
10-14-17-13- 8-20-22-18-12-17-11-18-21- 0-13-13- 7- 7		75.2	
13-13-21-10- 6-22-15-20-13-14- 7-20-12- 8-16- 9- 5-12		73.1	
Average 62.6			1.79657
pH 6.9			
10-19-19-17-22-18-14-26-17-23-23-19-15-10-23-17-18-17		101.7	
11-15-24-16-24-23-13-27-20-23-23-19-18-13-21-23-18-22		109.8	
2-15-15-10-18-15- 8-12-15-21-22- 4-10-10-20-15-12-13		72.8	
0- 5-13- 7-15-12- 3-13-13-16-16-10- 8- 9-15-13-13-11		59.7	
18- 9-18-18-12-15-10-10-10-15-15-10-10-20- 5-13- 8-19		73.1	
22- 2-16-13-12-18- 8-10-10-12-11-13-10-22-15-13-10-20		71.5	
13-10-25-18-17-27-20-28-19-16-22-25-13-22-13-28-16-17		108.5	
10- 3-17-16-17-23-14-15-18-16-16-21-19-23-12-21		92.1	
10-12-25-13-14-16-27-17-13-17-18- 8-13- 7-24-13- 6- 0		78.7	
10-10-24-13-16-21-16- 7-27-17-17- 8-15- 5- 7- 0- 0- 0		73.7	
7- 3-13-22-28-14-13-30-20-17-18-25-13-17-20-16-21-23		107.9	
16-19-12-16-24-10-13-20-20-17-18-25-10-10- 7-23-13-20		91.1	
15-13-13-29- 8-19- 6-20-17- 8- 9-23-16- 0- 4-15-10-11		71.8	
18-18-12-15-26-11-10-23-15-14-14-24- 8-15-10-17-10-19		86.8	
Average 85.0			1.92942
pH 7.9			
17-10-17-13-15- 8-12-15-10-10- 5- 3-20- 5-10-12-12-13		61.2	
10-13- 3-10- 7- 7-10-13-10-10- 0-10-15- 8-13-14-17-13		56.9	
12-13- 6-14- 7-17-15-12-15-10- 5-13-17-20-13-12-12-16		70.9	
15-10-18-10-10-10-17- 3- 5- 0- 5-10- 3- 0- 0- 0- 4- 5		32.7	
16-15-19-13-16-19-12-13-10- 6-11-13-18- 7-20-11-22-22		84.9	
15-13-22- 8-20-12-12-13- 0- 6-19- 5- 5- 0- 0- 0- 2-11		50.7	
16-11-23-10-15- 0-25-10- 3-17-17- 8- 8- 0-15- 0- 9- 9		57.5	
12-13-10-10-13-17-13-12-10-13- 2-13-17-10-10-10-10-10		63.8	
24-13-21-18-20-15-23-20-10-20-12-15-23-12-15-13-17-10		96.7	
21-13-23-10-20-19-21-17-14-17-17-13-23-15-16-14-20-13		95.4	
16- 9-18-17-15-15-20-17-11-15-13-11-18-11-10-11- 8- 0		73.1	
23-14-13-20-20-23-20-23-12-22-13-17-20-16-17-14-13-16		101.4	
17-21-17-18-24-16-19-10-18-17-17-19-17-14-13-16-23		96.4	
12-15-18-15-14-23-13-12-15-13-17-20-13-12- 0-25-16- 9		81.5	
10-13-13-14-13-13-12-12-13-13-14-23-13-12- 8-17-13-14		77.7	
15-18-20-14-23-25-12-13-10-20-16-19-20-21-11-23-16-14		99.6	
Average 75.8			1.87967



TABLE 3 (Continued).—Rate of root hair elongation in calcium chloride, 0.020 molar

pH 8.9	Average	Log.
2-11-12-10-10-17-13-13-13- 9-15-13-10-14-13-13- 0-14	62.8	
16-14-20-16-11-13-10-16-10-17-20-20-15-20-19-17-23-17	89.3	
13-10-20-13-20-13-12-17-11-19-18-20-12-15-20-13-14-23	88.0	
5- 9-13-13-17-17-19-17-14-13-16-19-17-18-11- 7- 4-27	77.7	
13-13-14-21-18-21-17-22-13-15-20-17-16-21-17-17-23-17	98.6	
13-14-13-13-20-17-13-17-16-17-10-22-21-17-17-18-22-20	96.4	
13-13-17-20-20-17- 6-17-13-10-17-13-13-24- 3-13-27-10	82.7	
12-12-13-13-20-17- 0-20-15- 8-20-20-10-17-20-13-34-10	85.2	
10- 3-24-10-13-13- 0-14- 8	59.1	
5- 3-23- 4- 7- 0- 0-13-10	46.7	
23- 6-14-10-13-13- 0-17- 7	70.3	
13- 3-20- 4-10- 7- 0-13- 8	48.5	
10- 0-18- 2- 8-12- 0- 7- 8	40.5	
17- 3-20-10-13-14- 5-10-10	33.5	
18- 5-17-10-13-15- 2-12-13	65.3	
15-14- 0- 3- 3- 2- 8-10- 5	31.1	
	-----	
Average	73.5	1.86629
pH 9.9		
10- 5- 3- 9- 3- 0- 7- 8- 0	29.8	
10- 7- 6- 2- 8- 0- 7- 3-10	33.0	
10-10- 3- 0- 4- 8- 2- 3- 5	28.0	
4- 4- 4- 7- 3- 5- 5- 0- 4	22.4	
3- 2- 5- 0- 0- 3- 2- 0- 3- 5- 2- 3- 3- 2- 2- 0- 3- 3	12.7	
3- 0- 3- 2- 5- 0- 3- 3- 7- 3- 2- 8- 2- 6- 5- 2- 5- 8	20.5	
4- 0- 3- 0- 3- 3- 2- 0- 5- 3- 4- 5- 2- 6- 5- 2- 7- 9	19.6	
0- 3- 2- 0- 2- 0- 0- 0- 3- 2- 0- 2- 8- 2- 3- 5- 5- 8	14.0	
0- 0- 3- 2- 0- 0- 2- 0- 0- 0- 0- 0- 0- 2- 3- 2- 0- 3	7.2	
	-----	
Average	21.0	1.32222
pH 10.9		
0- 0- 0- 4- 4- 4- 2- 1- 2- 3- 4- 3- 8- 7- 8- 9- 9- 9	22.7	
1- 1- 1- 7- 7- 6- 4- 4- 4- 5- 5- 5- 9- 8- 9- 8- 8- 8	31.1	
1- 1- 1- 7- 7- 6- 4- 4- 4- 5- 5- 5- 9- 8- 9- 8- 8- 8	31.1	
3- 3- 3- 2- 1- 2- 2- 1- 2- 0- 0- 0- 0- 0- 0- 0- 0- 0	5.9	
2- 1- 2- 2- 1- 2- 2- 1- 2- 0- 0- 0- 0- 0- 0- 0- 0- 0	4.0	
2- 2- 2- 0- 0- 0- 3- 4- 3- 3- 4- 3- 4- 4- 4- 2- 1- 2	10.3	
2- 3- 2- 0- 0- 0- 1- 0- 1- 2- 1- 2- 2- 3- 2- 3- 2- 3	9.0	
2- 2- 2- 0- 0- 0- 3- 2- 3- 2- 1- 2- 1- 0- 1- 0- 0- 0	6.5	
2- 2- 2- 0- 0- 0- 0- 0- 0- 0- 0- 0- 0- 0- 0- 0- 0	2.0	
	-----	
Average	11.3	1.05308
pH 11.4		
1- 2- 0- 0- 0- 0- 0- 0- 0- 1- 0- 0- 0- 0- 0- 0- 0- 0	1.7	
1- 1- 1- 0- 0- 0- 0- 0- 0- 0- 0- 0- 0- 0- 0- 0- 0	1.0	
1- 0- 1- 0- 0- 0- 0- 0- 0- 0- 0- 0- 0- 0- 0- 0- 0	0.8	
1- 1- 1- 0- 0- 0- 0- 0- 0- 0- 0- 0- 0- 0- 0- 0- 0	1.0	
	-----	
Average	1.1	0.04140

A graph based upon the data for 0.020 *M* CaCl<sub>2</sub> solution would be of very nearly the same form as that for 0.008 *M*, except that the upper portion of the curve is narrower, whereas the base embraces the same range, namely eight pH units. But the graph is shifted approximately one pH unit in the acid direction, so that the acid limit is now below 3.9 instead of above 4.9, and the alkaline limit is at 11.4 instead of 11.9. The top of the curve is of the same shape. However it will be noted that if the first 8 root hairs only in the pH 8.9 solution be considered, since the other 8 are on a different root, which apparently was not behaving normally, the average for this pH value would be 85.1, instead of 73.5. This would result in a bimodal curve with the alkaline and acid maxima the same. That this is more nearly correct is indicated by a study of an 0.028 *M* solution.

TABLE 4.—Rate of root hair elongation in calcium chloride, 0.028 molar

pH 3.4																		Average	Log.
0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0	
(16 hairs measured)																		0	0
pH 3.9																			
0-	0-	2-	2-	2-	1-	0-	0-	0-	0-	2-	1-	0-	0-	0-	0-	2-	2-	1	4.7
0-	0-	2-	1-	1-	1-	0-	0-	0-	0-	1-	1-	0-	0-	0-	0-	0-	0-	0	2.2
2-	3-	4-	4-	5-	4-	1-	0-	0-	2-	3-	2-	1-	1-	1-	2-	1-	1	14.6	
2-	1-	2-	2-	1-	5-	5-	5-	1-	1-	4-	3-	3-	3-	4-	3-	3-		18.7	
4-	3-	2-	3-	2-	3-	2-	2-	2-	3-	4-	2-	2-	2-	1-	1-	1-	1	14.0	
4-	3-	2-	3-	2-	3-	1-	1-	1-	0-	0-	0-	0-	0-	0-	0-	0-	0	6.2	
5-	5-	2-	3-	2-	3-	1-	1-	1-	0-	0-	2-	1-	1-	1-	1-	1-	0	9.3	
2-	2-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0	1.3	
2-	1-	1-	0-	1-	0-	0-	0-	0-	0-	0-	1-	1-	0-	0-	0-	0-	0	2.2	
3-	3-	2-	3-	2-	3-	2-	3-	2-	1-	1-	2-	2-	2-	1-	1-	1-	0	10.9	
5-	6-	2-	1-	2-	1-	0-	0-	0-	0-	0-	1-	1-	1-	0-	0-	0-	0	5.9	
1-	1-	0-	0-	0-	0-	0-	0-	1-	1-	1-	0-	0-	0-	0-	1-	1-	1	2.5	
0-	0-	2-	3-	2-	3-	0-	0-	0-	0-	0-	2-	1-	1-	1-	0-	0-	0	5.6	
																		Average	8.3
																		0.91910	
pH 5.9																			
12-	10-	11-	12-	2-	25-	7-	13-	12-	10-	18-	17-	15-	15-	10-	13-	7		70.6	
7-	8-	12-	8-	12-	10-	7-	10-	2-	15-	15-	13-	14-	6-	7-	13-	7-10		55.0	
3-	10-	4-	8-	7-	16-	7-	13-	15-	17-	5-	13-	14-	3-	5-	5-	10-10		51.3	
24-	3-	17-	0-	10-	15-	2-	10-	10-	6-	3-	4-	13-	7-	5-	0-	0		33.9	
12-	8-	12-	0-	13-	10-	12-	2-	10-	10-	16-	17-	5-	13-	5-	10-	0-2		42.6	
21-	10-	13-	0-	10-	13-	14-	8-	15-	3-	14-	7-	6-	13-	13-	9-	12-5		55.0	
16-	5-	18-	0-	7-	10-	17-	0-	12-	0-	15-	0-	0-	7-	18-	7-	3-5		40.8	
18-	12-	13-	10-	12-	15-	15-	10-	13-	14-	8-	17-	18-	17-	13-	10-	15-2		64.9	
11-	8-	9-	12-	10-	8-	17-	8-	9-	13-	20-	13-	14-	13-	10-	10-	13-15		63.1	
15-	10-	13-	12-	10-	15-	13-	12-	12-	13-	20-	15-	15-	13-	12-	12-	13-10		73.1	
12-	5-	13-	7-	10-	10-	15-	8-	10-	12-	12-	13-	13-	13-	12-	5-	18-12		60.0	
15-	9-	5-	13-	13-	12-	12-	13-	17-	17-	22-	11-	10-	15-	13-	12-	10-13		72.2	
19-	10-	12-	16-	14-	8-	18-	17-	15-	2-	29-	16-	15-	13-	10-	17-	13-13		79.9	
10-	7-	13-	13-	10-	12-	12-	20-	16-	9-	15-	20-	20-	5-	15-	13-	18-10		73.1	
6-	9-	8-	10-	7-	8-	12-	5-	25-	10-	7-	18-	18-	14-	15-	15-	18-12		71.5	
																		Average	60.7
																		1.78319	



TABLE 4 (Continued).—Rate of root hair elongation in calcium chloride 0.028 molar

pH 9.4										Average	Log.	
13-15-20-	8-12-16-10-11-21-22-10-10-25-15-13-17-10-20									84.9		
17-17-23-10-14-19-14-15-20-20-23-20-22-16-19-12-19-19									99.2			
18-15-17-	8-15-16-10-11-21-22-17-18-17-16-13-19-15-16									86.8		
20-15-13-	5-11-21-12-13-14-14-14-13-23-13-11-13-13-22									80.9		
20-13-17-	3-13-19- 4- 4-21-22-16-15-19-12-13-16-10- 9									73.1		
14-13-23-	5-14-18-12-13-14-14-17-18-25-12-12-13-13-14									82.1		
18-17-15-	8-13-14-16-17-15-15-18-19-20-16-14-10-10-16									84.3		
8-	0-11-10- 3-13- 0- 0- 6- 7- 2- 2									31.1		
10-	2-10- 5- 5- 8- 2- 3- 6- 6- 2- 5									28.9		
12-	0-11-10- 3-10- 2- 3- 5- 6- 3- 4									32.0		
13-	0-13-10- 3- 9- 6- 6- 8- 8- 6- 7									41.4		
10-	0-10- 4- 8- 5- 6- 7- 8- 9- 2- 3									33.6		
16-	0-14- 7- 7- 7-10-10- 2- 3- 6- 7									41.1		
7-13-	2- 3-10- 7- 3- 4-11-12-10-10									43.2		
10-12-	2- 8- 5-10- 6- 7-12-13- 7- 8									46.7		
										Average	59.3	1.77305
pH 10.4												
17-	5-10-10-10- 7- 8- 5- 5- 8-10- 6- 6- 5- 5-12-12-11									47.3		
16-10-	9-10-10- 9- 9- 6- 6- 8- 7- 5- 5- 5- 5-10-10-10									49.8		
20-13-12-10-10-	7- 8- 4- 4-13- 7- 6- 6- 5- 5- 9- 9- 9									49.1		
18-	7-13-10- 3- 6- 6- 6- 6-11- 2- 6- 7- 7- 7- 8- 9- 9									47.0		
21-13-13-10-	7- 3- 4- 5- 5-11- 2- 6- 7- 3- 4- 7- 8- 8									39.5		
21-13-13-	7- 7- 5- 5- 7- 6- 3-10- 6- 6- 1- 1- 8- 9- 9									42.6		
0-	0- 3- 7- 8- 8- 9- 6- 6- 8- 8- 6- 7- 5- 6-12-12-12									44.5		
4-	2- 3- 0- 0- 0- 0- 0- 0- 0- 0- 0									5.0		
6-	1- 1- 0- 0- 5- 3- 1- 2- 0- 0- 0									10.0		
0-	1- 1-13- 0- 3- 4- 0- 0- 2- 1- 0									13.0		
3-	0- 0- 0- 0- 0- 0- 5- 5- 2- 3- 0									9.0		
0-	1- 1- 0- 0- 3- 3- 0- 0- 2- 2- 0									7.0		
0-	2- 3- 2- 0- 0- 0- 4- 4- 4- 4- 0									12.0		
0-	1- 1- 0- 0- 0- 0- 0- 0- 0- 0- 0									1.0		
0-	2- 3- 0- 3- 0- 0- 0- 0- 0- 0- 0									4.0		
										Average	25.4	1.40403
pH 11.4												
0-	0- 0- 0- 0- 0- 0- 0- 0- 0- 0- 0- 0- 0- 0- 0- 0									0		
(10 hairs measured)										Average	0	0

The graph representing the data given above for growth of root hairs of *Brassica* in 0.028 *M* CaCl<sub>2</sub> solution is almost identical with that for 0.020 *M* both as to form of the curve and the pH range which is covered. The logarithmic curve especially shows marked median minimum at 7.9, which is exactly where the median minimum falls in the curve for 0.020 *M*, modified as suggested above giving it an alkaline optimum by the elimination of inappropriate data. The chief difference between the

graph for 0.028 *M* and for 0.020 *M* is that the latter is a little lower in height, that is, the maximum growth rate is a little less in the higher concentration. That this is logical is shown by the data given below for 0.060 *M*, in which the rate is markedly retarded. Had data been taken in the 0.028 *M* solution at pH 6.4 or 6.9 it is likely that a definite acid optimum would have been found, approximately equivalent to the alkaline optimum at 8.9. It is significant that the acid and alkaline optima are one pH unit more acid for 0.028 and 0.020 *M* solutions than for 0.008 *M* calcium chloride or for calcium hydroxide.

TABLE 5.—Rate of root hair elongation in calcium chloride, 0.060 molar

pH 3.4																	Average	Log.	
2-	1-	1-	1-	1-	0-	0-	0-	0-	0-	1-	1-	1-	2-	1-	1-	1-	2	6.1	
																	---		
Average																	6.1	0.78533	
pH 3.9																			
3-	3-	6-	6-	6-	8-	9-	8-	2-	1-	1-	5-	6-	4-	3-	6-	7	28.9		
6-	7-	6-	6-	6-	6-	9-	9-	6-	6-	6-	5-	5-	5-	5-	5-	6-	7	35.1	
0-	0-	6-	6-	2-	3-	0-	0-	2-	3-	2-	2-	3-	3-	2-	3-	4-	4	14.0	
3-	3-	2-	2-	2-	3-	6-	6-	2-	2-	2-	3-	4-	3-	2-	3-	4-	4	20.2	
2-	3-	5-	5-	6-	7-	3-	4-	6-	6-	6-	3-	4-	3-	10-	2-	5-	5	24.3	
6-	7-	0-	0-	7-	3-	1-	0-	1-	3-	4-	3-	2-	3-	1-	2-			13.4	
6-	7-	5-	5-	6-	4-	0-	1-	2-	2-	4-	4-	4-	6-	7-	0-	0		19.6	
1-	2-	0-	0-	1-	2-	5-	5-	3-	4-	3-	3-	4-	3-	8-	9-	0-	0	21.8	
5-	6-	7-	6-	7-	6-	6-	6-	4-	4-	4-	4-	4-	4-	1-	1-	0-	0	29.9	
5-	6-	4-	4-	2-	1-	2-	2-	0-	0-	0-	3-	4-	3-	2-	3-	1-	1	14.0	
5-	5-	2-	3-	7-	8-	2-	3-	2-	3-	2-	2-	3-	3-	2-	3-	1-	2	18.0	
4-	4-	3-	4-	0-	0-	1-	1-	3-	7-	7-	2-	3-	2-	2-	3-	0-	0	12.4	
5-	1-	2-	0-	0-	4-	5-	3-	4-	3-	1-	2-	2-	0-	0-	1-	2		12.4	
4-	4-	1-	1-	2-	3-	1-	2-	3-	4-	3-	1-	1-	1-	1-	1-	1-	1	13.1	
2-	2-	2-	3-	2-	2-	4-	4-	2-	3-	2-	3-	4-	3-	1-	2-	0-	0	13.4	
5-	5-	0-	0-	0-	0-	0-	0-	0-	0-	0-	3-	4-	3-	0-	0-	0-	0	6.2	
3-	4-	1-	2-	1-	2-	0-	0-	3-	4-	3-	3-	4-	3-	1-	2-	1-	1	11.8	
5-	5-	1-	2-	3-	4-	1-	2-	4-	4-	4-	1-	2-	4-	4-	4-	1-	1	14.6	
0-	0-	2-	3-	4-	4-	1-	2-	3-	3-	3-	1-	2-	2-	3-	4-	0-	0	11.5	
0-	0-	0-	0-	2-	2-	4-	4-	3-	4-	3-	1-	2-	2-	5-	5-	0-	0	11.5	
1-	1-	5-	5-	1-	2-	3-	4-	2-	3-	2-	2-	3-	2-	5-	5-	2-	3	15.6	
20-	30-	30-	30-	1-	2-	0-	0-	2-	1-	2-	5-	5-	5-	4-	4-	3-	4	15.6	
																	---		
Average																	16.2	1.20952	

TABLE 5 (Continued).—Rate of root hair elongation in calcium chloride, 0.060 molar

pH 4.4														Average	Log.	
7- 8- 5- 2- 8- 7-10-13- 0- 7- 5- 5-13- 4-13-10- 5- 2	38.6															
2- 3- 2- 8- 5- 5-15-10- 4- 8- 5- 5- 3-10-12-10- 0- 5	31.7															
3- 0- 1- 1- 1- 4-10- 3- 3-12- 3- 7- 7- 6-15- 2- 3- 2	23.3															
2- 0- 1- 1- 1- 5- 7- 0- 8-12- 3- 7- 7-10- 8-11- 4- 3	28.0															
10- 2-10- 8- 8-10- 3- 9-10- 4-11-10- 3- 5- 7- 0-10- 5	38.8															
7- 7-10- 7-10- 8- 3- 9- 9- 2- 7- 8- 0- 4- 8- 0- 5- 8	34.2															
7- 7- 8-10-10- 7-10- 7-11- 5-10-10- 3- 9- 2- 3-10- 8	39.9															
7- 3- 4- 5- 8- 5- 2- 8- 6- 6- 6- 9- 0- 5- 8- 0- 2- 3	27.0															
9-13- 8- 8- 9- 8- 7- 3-10-10- 7- 7-13-10- 8-10- 5- 4	46.3															
7-10-10- 7- 5-10- 7- 6- 9-10- 5- 8-10- 5- 7- 5- 8- 7	41.7															
5-10- 8-10- 5- 8- 9- 2-13- 0- 3-10- 7- 7- 8- 5- 2- 8	40.5															
5-10- 5- 8- 5- 8- 7- 4-11- 5- 7- 6-11- 3-10-10- 5- 8	39.9															
5- 9- 6- 8- 8- 3- 5- 7-10-10- 3-10-10-10- 7- 5- 5- 7	42.6															
5- 7- 5- 5- 8- 2-13- 7- 0-10-10-10- 4- 9-10- 4-10- 3	44.2															
5- 5- 3-10- 2-10- 6- 7- 3- 9- 5- 7- 3-10- 5- 5- 6- 9	34.2															
2- 3- 2- 2- 8- 3-12- 3- 7- 3- 7- 5- 0- 5- 7- 0- 7- 3	24.9															
4- 6- 9- 3-15- 7-10- 6- 9-13-10- 5-10- 7- 3-15- 6-10	45.7															
9- 3- 9- 5-11-10- 7- 8- 9- 7-10-10- 5- 9-10-10-10- 8	44.2															
														Average	37.0	1.56820
pH 5.4																
4-10- 3- 8- 5-12- 0-10- 3- 5- 2- 8-10- 3- 2-10-12-10	42.6															
6- 7- 3- 5- 5- 3- 7- 7- 5- 2- 6- 4- 6- 7- 0-13- 5- 7	33.6															
6-10- 3- 9- 4- 3- 5- 5-15- 5- 2- 8- 5- 5- 5-10-15- 5	37.3															
7- 1- 8- 5-10- 4- 8- 3-12- 2-10- 8- 5-10-10- 0- 5- 2	34.2															
9- 6- 6- 9-10- 5-10- 8- 8- 9-12- 3-10-10-10- 0- 7- 8	43.6															
10- 3- 9-10-10- 5-10- 6- 9- 4- 8-11- 5-10-10- 0- 7-10	42.0															
5- 8- 5- 7-10- 6- 9- 2-10- 8- 8- 7- 6- 9- 8- 0- 4- 3	35.8															
3- 7- 3- 7- 4- 6- 2- 2- 8- 5- 3- 5- 5- 3- 9- 0- 2- 0	23.0															
1- 0- 3- 7- 3- 3- 9- 3- 7- 2- 6- 7- 0- 3- 0- 5- 2- 3	22.7															
7- 4- 7- 3- 5- 5-10- 4- 8- 2- 6- 7- 0- 6- 4- 3- 3- 4	28.0															
8- 5- 3- 7- 3- 3- 9- 5- 5- 3- 7- 0- 8- 2- 3- 0- 7- 5	25.8															
5- 3- 4- 5- 4- 6- 8- 7- 3- 7- 3- 7- 3- 2- 2- 2- 4- 3	24.6															
														Average	32.8	1.51587
pH 6.4																
7- 3- 3- 9- 2- 8- 2-11- 6- 6- 5- 3- 2- 7- 2- 8- 3- 2	30.2															
4- 3- 4- 9-10- 3-10- 6- 6-13- 2- 8- 5-10- 3-10- 5- 9	37.6															
10- 2- 8- 7-10- 5- 9- 9-10- 7- 8- 5- 7-10- 6- 9- 5-10	42.6															
8- 7- 3-10- 3- 4- 7- 6- 7- 8- 2- 3- 7- 6- 7- 7- 5- 5	31.1															
9- 5- 7- 3- 8- 5- 5- 5- 5- 2- 5- 3- 5- 2- 0- 0- 0- 0	21.5															
9- 8- 6- 2- 7-12- 7- 3- 7- 3- 3- 2- 5- 2- 0- 1- 0- 0	22.7															
11- 7- 7- 6- 4- 8- 7- 8- 6- 9- 8- 7-10- 3-10- 3- 7- 0	37.6															
2- 3- 7- 3- 7- 8- 2- 5- 5- 3- 2-10-10-10-10- 1- 7- 5	31.1															
10- 7-10- 5- 5- 5- 3- 5- 8- 2- 5- 2- 2- 7- 2- 3- 0- 2	25.5															
8- 7-10- 7- 3-10- 3-10- 3- 9- 2- 8- 3-10- 2- 3- 7- 0	32.7															
10- 5-13- 6- 5- 8- 5-10- 6- 7- 3- 9- 2-11- 2- 8- 5- 0	36.7															
2-10- 3- 7- 8- 7- 5- 7- 5-10- 7- 5- 4- 6- 5- 8- 2- 0	34.8															
2-10- 0- 8- 8- 9- 2- 8- 5-10- 0- 3- 7- 6- 0- 9- 2- 0	27.7															
0-10- 0- 8-10- 9- 0-10- 7- 1- 7- 3- 7- 7- 5- 8- 2- 5	31.1															
3- 6- 4- 6- 9-12- 3-10- 3-10-10- 2-10- 8- 7-10- 3-12	42.0															
														Average	32.5	1.51188

pH 10.4

0- 0- 0- 0- 0- 0- 0- 0- 0- 0- 0- 0- 0- 0- 0- 0- 0	0
(10 hairs measured)	Average 0 0

The graph based upon this data of 0.060 *M* CaCl<sub>2</sub>, is likewise steep sided, and markedly bimodal. In this case the acid optimum, 4.4, and the alkaline optimum, 8.4, are farther apart on the pH scale than in any other solution studied. The range has moved still farther toward the acid side, extending from 3.4 to less than 10.4. This shifting of the pH range of the solution toward the acid seems definitely related to the molar concentration of the salt. It may very well be due to an antagonism of the calcium ion for the toxic effect of the hydrogen ion. This will not, however, explain the shifting of the alkaline limit. The latter may be due to the additive injurious effects of the chloride and hydroxyl ions.

TABLE 6.—Rate of root hair elongation in calcium chloride, 0.120 molar

pH 3.4																		Average	Log.	
0-0-0-0-0-0-0-0-0-0-0-0-0-0-0-0-0-0																		0		
(no aquatic root hairs formed)																		---		
																		Average	0	0
pH 5.9																				
2-3-2-0-0-0-1-2-1-4-5-4-2-3-0-0-2-3																		10.0		
1-0-1-0-1-0-0-0-0-0-0-0-1-0-0-0-0-0																		4.7		
1-0-0-1-1-1-0-0-0-0-0-0-3-4-3-5-5-0-0																		7.5		
0-0-0-0-0-0-1-0-1-0-0-0-4-4-4-6-6-0																		10.0		
0-1-0-1-0-0-0-0-0-2-1-2-3-2-6-7-0-0																		8.4		
0-0-0-0-0-0-0-0-1-1-1-1-1-1-6-7-0-0																		6.0		
2-3-2-3-2-0-0-0-0-0-0-0-0-0-0-0-0-0																		3.7		
3-3-3-3-3-0-0-0-2-3-0-0-0-2-3-0-0-0																		7.8		
2-0-2-2-2-0-0-0-3-4-3-0-0-0-2-3-2-3																		13.4		
3-3-3-3-3-0-0-0-3-4-3-0-0-0-1-1-0-0																		8.4		
3-4-3-3-3-2-3-2-1-2-1-0-0-0-0-0-1-1																		10.8		
4-0-4-4-4-0-0-0-1-0-1-0-0-0-1-2-1-2																		7.5		
2-2-2-2-2-2-1-2-4-3-4-4-3-4-4-1-2																		16.2		
4-0-4-4-4-1-2-1-2-1-2-3-4-3-3-3-5-6																		16.2		
4-3-4-4-4-2-2-2-4-3-4-3-2-3-4-3-4-3																		16.9		
4-3-4-4-4-2-3-2-3-3-3-2-2-2-3-4-1-2																		16.9		
3-0-3-3-3-3-4-3-3-3-3-2-3-2-1-1-5-5																		15.6		
3-2-3-2-3-1-2-1-2-3-2-3-4-3-5-5-1-2																		15.2		
3-4-3-4-3-2-3-2-3-3-3-3-5-5-2-3-2-3																		17.4		
1-2-0-1-2-3-4-3-2-2-2-2-1-2-0-0-6-7																		11.8		
0-0-0-0-0-2-2-2-4-5-4-1-2-2-2-3-7-8																		12.7		
1-0-1-1-0-1-6-5-6-1-0-1-1-0-1-2-2-2																		10.0		
0-1-0-0-1-0-5-6-5-1-0-1-1-2-1-3-4-3																		11.8		
2-1-2-2-2-3-2-3-3-2-3-3-5-5-2-2-2																		14.3		
																		Average	11.0	1.04139





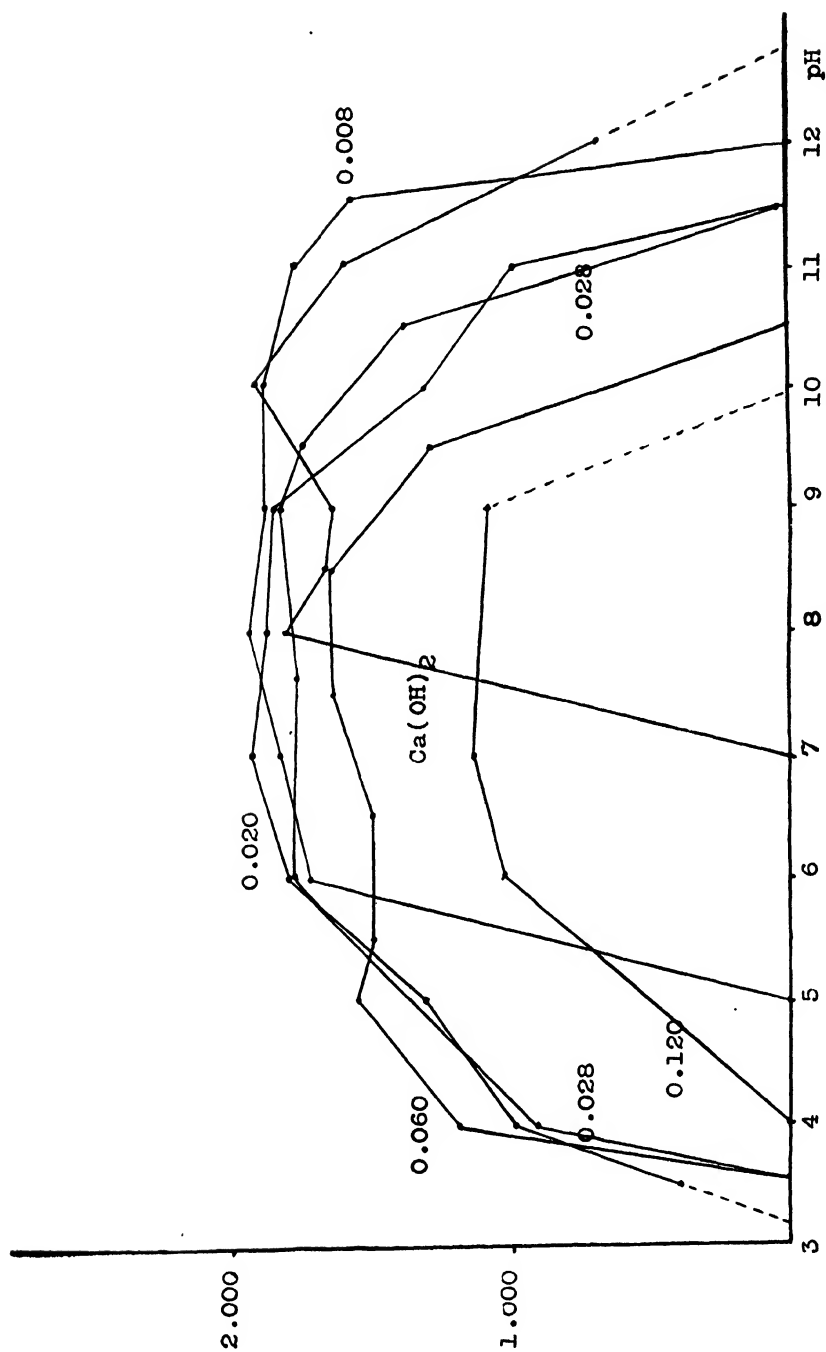


FIGURE 1.—Graphs representing the common logarithms of the rate of elongation of root hairs of Georgia collards in solutions of different molar concentrations of calcium chloride over a range of hydrogen ion concentrations, including the graph for calcium hydroxide

## GRAPHICAL REPRESENTATION OF THE RATE OF ELONGATION

From the data given in Tables 1 to 6 graphs may be constructed which will represent the rate of elongation of these root hairs in various hydrogen ion concentrations for the respective molar concentrations of the salt used. These graphs may be based upon the maximum rate of elongation in each solution, that is, the rate of the most rapidly growing hair; or they may be based upon the average of all of the hairs measured in the given solution, or they may, finally, be based upon the logarithms of these averages. In Fig. 1 graphs of the last-named types are given. In Fig. 2 the graphs based upon the averages are used. The two sets of graphs show no essential differences, except that the median variations are less in the logarithmic curves.

In Fig. 2 there is represented a three-dimensional graph based upon the average rate of growth in each of the solutions studied. The light-colored graph in the foreground represents the data for calcium hydroxide. The darker graphs with white stripes represent the concentrations of calcium chloride ranging from 0.008 *M* in front to 0.120 *M* in the distance. On the graphs bearing three stripes, the middle one indicates the location of the median minimum on the pH scale. The left stripes respectively indicate the acid optima, and the stripe on the right-hand side the alkaline optimum. The graph for 0.120 *M* solution is monomodal and hence one optimum only is shown. This should doubtless be interpreted as the alkaline optimum. The white field, approximating the shape of a triangle represents the tolerance of this plant for molar and hydrogen ion concentrations of this salt. The heavy line along the middle of this field represents neutrality, pH 7. Those to the left and right of it indicate successive pH units below and above this respectively.

## INTERPRETATION OF RESULTS

If we look now for an interpretation of these results, we must conclude that the characteristic pH curve for the growth of root hairs of *Brassica oleracea* in all, except very high, concentrations, is very broad and distinctly bimodal. The explanation of the occurrence of a medium minimum cannot be the mutual precipitation of ions within the solution, inasmuch as only calcium, chloride, hydrogen and hydroxyl ions are present. Neither does it seem plausible to attribute it to the internal factor of the isoelectric point of the constituent proteins, inasmuch as the median minimum shifts markedly toward the acid side with increasing concentration. In calcium hydroxide it is at pH 8.9; and by the addition of the salt it shifts, so that in 0.060 *M* calcium chloride it is at pH 5.9; and in the intermediate concentrations it holds intermediate positions. It is inconceivable that such small changes in concentration of the

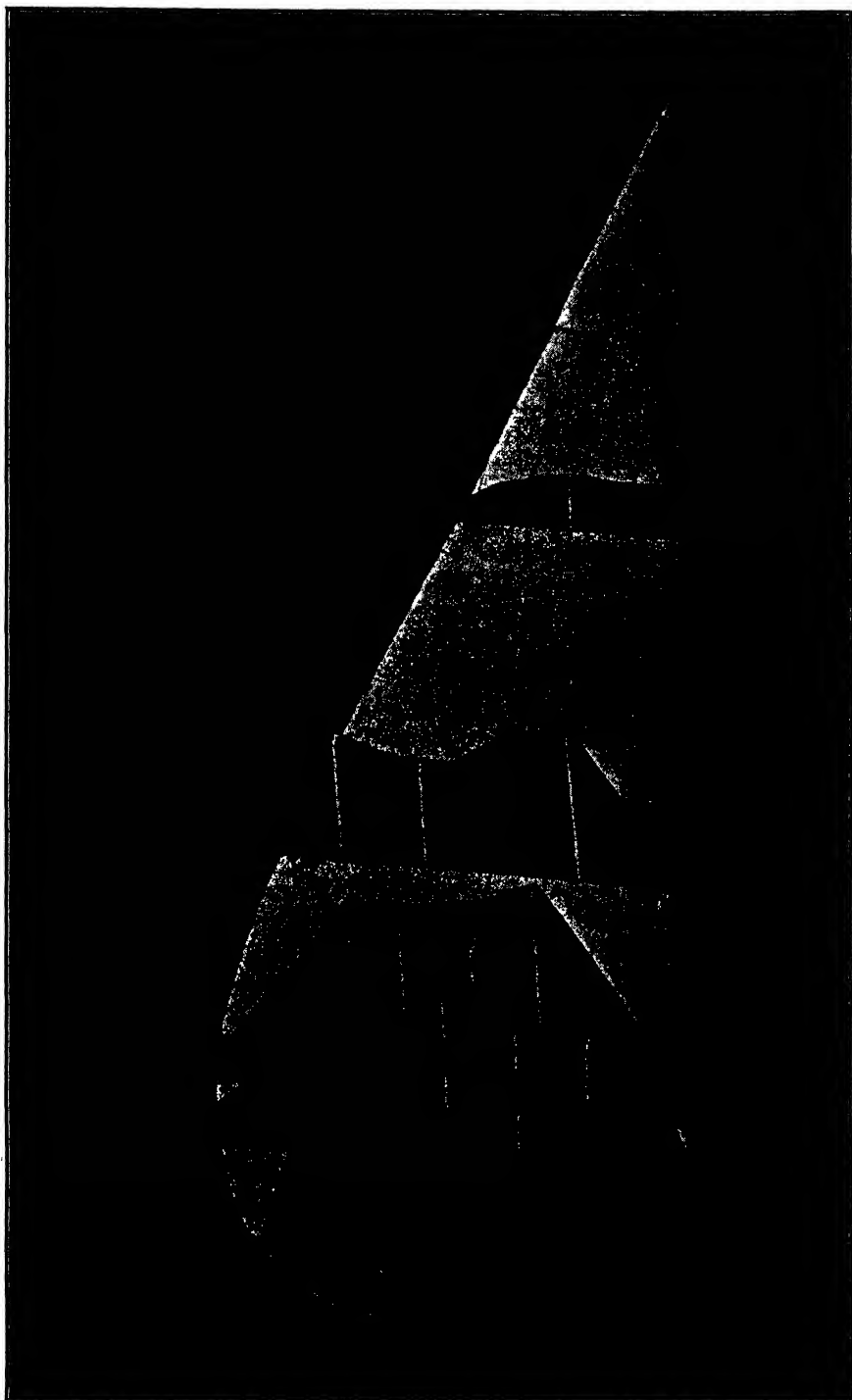


FIGURE 2.—Tri-dimensional graph based upon average rate of elongation of root hairs of Georgia collards in solutions of calcium hydrate and calcium chloride

medium would alter so fundamental a property as the isoelectric point of a chemical compound. No other explanation for the median minimum presents itself at present, except that it is the result of two optima, an acid and an alkaline. It is indeed possible to consider the median minimum as a feature merely incidental to the existence of two optima, that is, to two over-lapping curves. Upon this basis our attention must shift to an interpretation of the two optima.

The two optima also shift toward the acid side with increasing concentration of the salt. The alkaline optimum shifts from pH 9.9 to pH 8.4. The acid optimum shifts from pH 7.9 to pH 4.4. In like manner the alkaline limit shifts from pH 11.9 to pH 9.4. At low concentrations the acid limit also shifts from pH 6.9 to pH 3.4. It is apparent therefore that the effect of increasing concentration is to increase the tolerance of the root for acid, and to decrease the tolerance of the root for alkali.

An exception to this rule is, however, apparent with respect to the acid range in very high concentrations. Here an increase in concentration decreases the tolerance for acid. It appears that this is very likely due to the fact that under these circumstances the calcium salt is itself approaching a toxic concentration, as is shown by the decrease in maximum growth rate in concentrations above 0.020 *M* solution. At high concentrations then there is the combined toxic effect of the calcium and the hydrogen ions, causing cessation of growth when both are present in excess. This results in the elimination of the acid portion of the curve for very high concentrations, as 0.120 *M* for instance, leaving only the alkaline optimum and the alkaline range. Hence the monomodal curve.

If we conceive of pH 0 representing the absence of hydroxyl ions, and pH 14 representing the absence of hydrogen ions, and the intermediate pH values representing mixtures of hydrogen and hydroxyl ions, then we may conceive of the acid optimum in these graphs as indicative of a response of the root to an optimum number of hydrogen ions, and the alkaline optimum to an optimum number of hydroxyl ions. That is, there may be an optimum hydrogen ion concentration and also an optimum hydroxyl ion concentration. The acid optimum we may associate with the former, and the alkaline with the latter. The median minimum, then, becomes intermediate between the optimum hydrogen and the optimum hydroxyl concentration, representing a balance between the two ions which does not support so high a rate of growth as does either of the ions in optimum concentration.

The presence of any other ions, than hydrogen and hydroxyl, in the solution, will obviously modify the location of these two optima on the pH scale, as well as the limits of the range. We may look upon the shifting of the acid optimum with increased concentration of calcium chloride, as an effect of the calcium ion in making it necessary that more hydrogen ions be present in order to bring about the same rate of growth. Its effect upon the alkaline optimum, however, must on this basis be

attributed to the calcium ion making it necessary that there be fewer hydroxyl ions in order to produce the optimum result or the shifting of the alkaline optimum may also be attributed to an effect of increased chloride ion content. Above a certain concentration, namely pH 9.9, hydroxyl ions, if they are the only anions present, become increasingly toxic to root hair growth. If chlorine is added, this toxicity begins at a lower hydroxyl concentration, that is, at pH 8.9 or less. In other words, there appears to be a combined toxic effect of the chloride and the hydroxyl ions at increased salt and hydroxyl concentrations, in contrast to the antagonistic relation of calcium and hydrogen ions at moderate or low concentrations. At high concentrations, however, the calcium and hydrogen are likewise additively toxic.

By arranging the six graphs for rate of growth of root hairs in various hydrogen ion concentrations in solutions of calcium hydroxide and 0.008, 0.020, 0.028, 0.060, 0.120 *M* calcium chloride upon a base at distances apart commensurate with these molar concentrations, we can construct a three dimensional graph, the floor plan of which will present the tolerance of the plant for salt and hydrogen ion concentration. Such a floor plan will be in the form of an isosceles triangle, with the lower left (acid) corner truncated, the altitude being neutrality. This truncated corner represents the lethal effect of hydrogen ions in the absence of calcium ions, so marked a feature of soil science. It is exceedingly significant that the optimum conditions for root hair elongation lie very close indeed to this lethal zone. While pH 4.9 in 0.008 *M*  $\text{CaCl}_2$  is lethal, pH 6.9 in 0.020 *M*  $\text{CaCl}_2$  supports the most rapid growth of any solution so far studied. The change of the former into the latter solution is accomplished by the addition of a very small amount of lime. This study then simply serves to emphasize in a rather exact way the beneficial effects of adding lime to acid nutrient media low in that element. It also graphically presents the effects of continuing the addition of calcium beyond the optimum concentration or to acid media already high in that element. In the latter instance the calcium compound will have a directly retardative effect inasmuch as it will raise the molar concentration more rapidly than the pH value. Addition of lime or calcium salts to the optimum, however, will have little effect upon growth unless it be very much in excess. A small amount will lower the rate slightly to the median minimum; the addition of more will cause it to accelerate up to the alkaline optimum, and it is only after this point is passed that a steady decline will be experienced.

For practical purposes, then, the quantity of calcium added to the soil is not significant, provided it is above a certain minimum, except in nutrient media which are already and continue to be distinctly acid in reaction. In most cases the presence of phosphates and sulfates in the solution satisfactorily prevent the attaining of toxic concentrations by the formation of insoluble salts.

# THE SIGNIFICANCE OF SMALL AMOUNTS OF INORGANIC ELEMENTS IN PLANTS<sup>1</sup>

J. S. McHARGUE

*University of Kentucky, U. S. A.*

## INTRODUCTION

The economical production of an ample and continuous supply of nutritious food, maintaining in the meantime a high state of fertility in the soil is as yet an unattained goal in the art of agriculture.

During the three hundred years in which agriculture has been practised in this country, magnificent forests have been extravagantly destroyed and the fertility of virgin soils exploited and exhausted to such an extent that large areas of land in the eastern part of this country have been abandoned because important agricultural crops cannot be produced upon them. There are also much larger areas of soils whose fertility is maintained from year to year in a semi-productive condition by means of artificial and temporary methods of fertilization and cultivation. These facts remind us of the truth contained in the statement made by Xenophon more than two thousand years ago, who said "Agriculture is an art which will enrich those who diligently practise it, provided they understand it; but, if they do not understand it, it matters not how hard they may labor at it, it leaves them in poverty." This characterization of agriculture serves to impress upon us the little progress that has been attained in understanding and interpreting the underlying principles of the oldest and yet the most fundamentally important of the various industries created and developed by civilized man.

In recent years, however, we are beginning to understand that the production of quality in food is a problem of growing importance in the art of agriculture. It is a well-known fact that in the days of the pioneer, when virgin soils possessed their maximum natural fertility, foods of superior quality were produced as a natural consequence. It is also a matter of observation and experience that certain types of soil possess an inherent property of producing foods that undoubtedly have superior nutritional qualities. A very striking example of such a soil area as that in mind is the Blue Grass Region of central Kentucky. Mr. Benjamin G. Bruce, who was an authority on the breeding and rearing of fine live stock in central Kentucky, is the author of an interesting article entitled "The Influence of Climate and Soil on Animals" which was published in Perrin's

<sup>1</sup> Published by the permission of the Director of the Kentucky Agri. Expt. Sta.

History of Fayette County, Kentucky, in 1882. He said, "There is no region of America so highly favored for the breeding and rearing of the fine horse as the Blue Grass Region of Kentucky, of which Fayette County is the center. This is to be attributed, in a large degree, to the nature of the soil in which he is reared. There is no doubt that animals which are fed upon grass and grain, and drink water impregnated with lime, have larger and stronger bones than those raised upon clay and sandy soils. The best sections of the country to breed and rear fine stock will be found, as a rule, to follow the limestone formations of America. The Blue Grass Section of Kentucky, and that portion of Tennessee with a tier of counties surrounding Nashville, are strong limestone regions, which accounts for the celebrity and superiority acquired by the horses of these sections over less favored portions of America. The high, rolling land, the limestone water, the quantity and quality of the herbage, is the cause, in our opinion, of the success of Kentucky and Tennessee breeders. The climate is suitable to the constitution of the horse, and the land grows that description of provender so well calculated for his sustenance and development. . . . Succulent herbage tends to the production of fat, with loose, flaccid muscle, and sinews of a similar texture, the very reverse of that which is requisite for the thoroughbred horse, or indeed for any animal that is to be qualified for speedy or continued exertions; whereas in the Blue Grass Region of Kentucky the land is dry and sound, somewhat elevated and rolling, conducing to a clean, wiry and muscular animal.

"The influence of climate on the animal and vegetable kingdom, has not escaped the notice of philosophers, and many learned treatises have been written to show the operation of this cause. Another cause no less powerful in its effects on men, animals and plants, has been cooperating with climate, to modify all living things, which certainly has not attracted proper attention, the geological formations of the different portions of the earth. The attention of geologists and natural philosophers has been confined to the dead and buried, to the age of the earth, to mining, the formation of coal beds, engineering, and the nature of soils in their relation to production. We know of no one who has written in regard to the effects that are produced by geological formations on living things. Perhaps there is yet to come some scientist with the genius, patience and industry of Darwin, Tyndal, Huxley or Spencer who will explore this new and interesting field, unfolding the secret influence of geological formations on living things hid away in nature's laboratory."

"Animal formation is modified by the vegetable formations of which it is the result, and the vegetable formations are modified by the elements of the soil from which they derive their nourishment. Not only the forms of animals, but their physical systems, their secretions and excretions, are affected by the difference of geological formations from which they derive, through its vegetation, the elements of their organization."



It is of interest to note how the above almost prophetic conception which was recorded nearly 50 years ago coincides with the more recent idea concerning the possible functions of a number of the so-called non-essential elements which occur in small amounts in rocks, soils, plants and animals.

According to Washington, chemist and petrologist at the Geophysical Laboratory of the Carnegie Institution, Washington, D. C., 95 per cent of the earth's crust consists of igneous matter, which contains compounds of 44 different elements in different proportions. Dr. Washington has computed the average composition of the earth's crust from a large number of chemical analyses of igneous rocks from various parts of the surface of the earth. The results are contained in Table 1.

TABLE 1.—*The chief elements in the earth's crust in order of their abundance.*

	Per cent		Per cent
1—Oxygen	46.43	23—Lithium	0.003
2—Silicon	27.77	24—Copper	.002
3—Aluminium	8.14	25—Cerium	.001
4—Iron	5.12	26—Glucinum	.00XX
5—Calcium	3.63	27—Cobalt	.00XX
6—Sodium	2.85	28—Boron	.000X
7—Potassium	2.60	29—Zinc	.000X
8—Magnesium	2.09	30—Lead	.000XX
9—Titanium	.629	31—Arsenic	.000XX
10—Phosphorus	.130	32—Cadmium	.0000XX
11—Hydrogen	.127	33—Tin	.0000XX
12— <i>Manganese</i>	.096	34—Mercury	.0000XX
13—Fluorine	.077	35—Antimony	.0000XX
14—Chlorine	.055	36—Molybdenum	.0000XX
15—Sulfur	.052	37—Silver	.00000XX
16—Barium	.048	38—Tungsten	.00000XX
17—Chromium	.037	39—Bismuth	.00000XX
18—Zirconium	.028	40—Selenium	.000000XX
19—Carbon	.027	41—Gold	.000000XX
20—Vanadium	.021	42—Bromine	.000000XX
21—Nickel	.019	43—Tellurium	.0000000XX
22—Strontium	.018	44—Platinum	.0000000XX

It is of interest to note the order of the abundance in which certain of these elements, which have been commonly referred to from the standpoint of agriculture as the so-called non-essential elements, occur in the earth's crust. For example, the element manganese occurs 12th, in the order of abundance in the earth's crust. Fluorine is 13th, barium 16th, nickel 21st, strontium 22nd, lithium 23rd, copper 24th, cobalt 27th, boron 28th, zinc 29th, lead 30th, arsenic 31st, bromine 42nd. Iodine, which, according to Clarke, is less abundant in nature than bromine, is not given in the list, yet it is a well-known fact that iodine is quite widely distributed in small amounts in natural waters, plants and animals.

In the processes of erosion, weathering and disintegration of igneous rocks considerable areas of limestone strata have been formed, some of which, upon disintegration, have produced soils that possess unusual properties of fertility as evidenced not only by more luxurious growth and yield of crops but also by the quality of the crops which undoubtedly have superior nutritional properties, as is further shown in the production of more excellent strains of domestic animals. Such a soil area has resulted from the disintegration of the phosphatic Trenton limestone strata of central Kentucky. The fresh, unaltered rock of these strata contains about 0.2 of one per cent of phosphorus, 0.25 per cent of manganese and appreciable amounts of copper, zinc, nickel, cobalt, boron, barium, strontium, lithium, arsenic, bromine and iodine, which also occur even to a greater extent in some instances in the soil than they do in the parent rocks from which the soils have been derived.

For example, a fertile soil of the Blue Grass Region of Kentucky contains in 1 kg. of the dry matter 4,500 mg. of manganese, copper 7.2 mg., zinc 27.7 mg., nickel 4. mg., cobalt 1.5 mg., arsenic 2.14 mg., and barium 471 mg.

*Poa pratensis* (Kentucky blue grass) grows perhaps more luxuriantly in the soils of central Kentucky than anywhere else in the world. Analysis of a sample of this important forage crop shows that 1 kg. of the moisture-free grass contains 14. mg. copper, 320 mg. iron, 80 mg. manganese, 17 mg. zinc and traces of nickel, cobalt and arsenic. A sample of soybean seeds grown on a blue grass soil contained in 1 kg. 12 mg. copper, 70. mg. iron, 32.5 mg. manganese, 18.4 mg. zinc, 3.9 mg. nickel and a trace of cobalt. A number of samples of other forage crops, cereals and food materials which have been grown under natural conditions in the soils of this region show that small quantities of these elements occur in larger amounts in certain vital parts of the plants than others. For example:

	kg.	mg. Cu	mg. Mn	mg. Zn
Wheat bran contained in	1	16	125	75
Wheat germs	Do	46	150	160
Wheat flour	Do	trace	10	22
Kernels of cotton seed	Do	54	13	320
Germs of white corn	Do	12	28	224
Germs of yellow corn	Do	15	30	272
Rice polishings	Do	8	112	76
Polished rice	Do	trace	10	7.5
Oats	Do	30	58	89

Other results show that the young and tenderest parts of the foliage of plants contain appreciably more of these elements than do the more mature leaves, thus showing that larger amounts of these elements are associated with the most actively growing parts of the plant.

## DETERMINING WHETHER CERTAIN ELEMENTS ARE FACTORS IN PLANT GROWTH

The foregoing results and others as well have induced us to undertake the rather difficult task of ascertaining whether or not the elements manganese, copper, zinc, nickel, boron, etc., are necessary factors in the growth of plants. Time will not permit of my going into details of the methods used in obtaining our results; however, in brief it may be said that by means of specially made pots, purified quartz sand, purified plant nutrients and specially purified distilled water results have been obtained which convinced us that more than 10 elements are necessary for the growth of plants and that manganese, copper and zinc are apparently important factors in plants' economy.

As to the functions of these elements in the growth of plants it can be said that manganese is apparently concerned in the synthesis of chlorophyll because when this element is carefully excluded from sand cultures containing adequate amounts of available manganese-free compounds of the ten essential elements, plants grow until the nutrients contained in the seeds are exhausted, become chlorotic and finally die, but when manganese is added all other conditions being identical the plants grow in a more normal way and do not become chlorotic.

When the chlorotic leaves are analyzed they show a normal content of iron and a very marked diminution in the manganese content when compared with the results of analyses of leaves of plants grown in sand cultures to which manganese has been added.

Furthermore, a few samples of lime induced chlorotic plants have been analyzed for the purpose of comparing the results obtained with similar analyses made upon normal green leaves of the same species and the results are reported in Table 2.

*TABLE 2.—Iron and manganese content of normal and lime-induced chlorotic plants*

Description of plant material	Per cent	
	Fe	Mn
Chlorotic spinach leaves	0.1110	0.0017
Normal green spinach leaves	.0915	.0168
Chlorotic sugar cane leaves	.0241	trace
Normal green sugar cane leaves	.0358	.0300
Chlorotic pin oak leaves	.0377	.0090
Normal green pin oak leaves	.0358	.0552
Chlorotic corn plants	.0326	.0003
Green corn plants+manganese	.0357	.0239
Normal corn plants grown under field conditions	.0388	.0560

It is to be observed that in each sample of chlorotic leaves there is a marked diminution in the manganese content when compared with the results for this element in the normal green leaves whereas the iron content of the chlorotic leaves is equal to and in two instances (spinach and pin oaks) it is appreciably greater than it is in the normal green leaves. Moreover, when the live chlorotic leaves are tested for the presence of peroxidases there is a very marked diminution in the intensity of the reaction obtained when compared with the intensity of the reaction obtained on the green leaves produced as the result of the addition of manganese to the cultures, thus indicating in a rather definite way that this element is concerned in the oxidative processes taking place during the plant's growth.

The spinach plants were obtained through the courtesy of the Rhode Island Experiment Station. The chlorotic plants were produced after heavy applications of lime on soils which had a low manganese content. The chlorosis thus produced was cured by spraying the plants with a dilute solution of manganous sulfate. Spraying with iron salts did not have any curative effects. A similar type of chlorosis has been observed by Schreiner and Dawson on tomato plants grown on certain glade soils in southern Florida and the chlorosis was cured by the application of manganese. Iron salts had no effect in bringing about a cure on tomato plants.

The sugar cane plants were obtained through the courtesy of Dr. H. A. Lee of the Hawaiian Sugar Cane Growers' Association of the Hawaiian Islands, where they are having trouble with sugar cane becoming chlorotic when grown on certain types of soil. The application of manganese compounds has likewise brought about a cure of chlorosis in sugar cane but iron compounds have failed to do so.

The occurrence of copper in larger proportions in the germs of seeds is an indication of some important function in the growth of the plant, and this assumption is further supported by evidence obtained by means of carefully controlled experiments.

It is a well known fact that a Bordeaux spray has a beneficial effect on the growth of certain plants as well as controlling fungicidal diseases.

#### OCCURRENCE OF SMALL AMOUNTS OF INORGANIC ELEMENTS IN ANIMALS

Manganese, copper, zinc, nickel, cobalt, boron and iodine are found in the animal body. Manganese occurs in greatest concentration in marine and fresh water mollusks, some of which contain 5 times as much manganese as iron. It is reported by one investigator that this element has a respiratory function in the metabolism of the mollusks. Manganese is widely distributed in the tissues of higher animals, occurring in greater concentrations in such vital organs as the liver, kidneys, spleen,

pancreas, heart and brain. It occurs in even greater concentration in the commercial preparations of the digestive enzymes, pepsin, rennin and trypsin which is indicative of some important function in connection with these important enzymes.

Copper is a normal constituent of the animal body—a fact which has been known for more than 100 years. It occurs in larger amounts in the liver than any other organ. It occurs in greatest concentration in the embryos of mammals. The livers of young calves are unusually rich in copper. Appreciable quantities of copper occur in the yolks of eggs and in the cream of milk. Oysters are rich in copper and certain crustaceans contain hemocyanin, which has a respiratory function in their metabolism.

Zinc and boron are also widely distributed in the animal body.

Bertrand reports a concentration of nickel and cobalt in the pancreas of animals and claims that compounds of these metals have an effect similar to that of insulin in the metabolism of sugar in diabetics. If such is a fact then the very minute amount of nickel present in plants is of utmost importance in the normal metabolism of animals.

### SUMMARY

Since all food material is ultimately synthesized in the plants' economy it is therefore a matter of fundamental importance to know what elements are necessary for the plant's growth in order that we may better understand how to produce foods not only in quantity but also foods of the highest quality as well.

### LITERATURE CITED

- (1) Bertrand, G. 1926. The importance of minute chemical constituents of biological products; nickel, cobalt and insulin. *Science* 64, No. 1669.
- (2) Bruce, G. 1882. *Perrins History of Fayette County*, P. 131-2.
- (3) Gilbert, B. E., McLean, F. T., and Hardin, F. J. 1926. The relation of manganese and iron to a lime induced chlorosis, *Soil Sci.* 22, No. 6.
- (4) Schreiner, O., and Dawson, R. R., 1927. Manganese deficiency in soils and fertilizers. *Indus. Engin. Chem.*, 19: 400.
- (5) Washington, H. S. 1920. The chemistry of the earth's crust, *Jour. Franklin Institute*, 190: 757.

# THE SIGNIFICANCE OF NITROGEN IN SOIL ORGANIC MATTER RELATIONSHIPS

F. J. SIEVERS AND H. F. HOLTZ

*State College of Washington, U. S. A.*

## INTRODUCTION

The formation of soil, primarily involves the disintegration of the rock materials through the process of weathering. The first product from this process is therefore composed very largely of mineral matter, similar in composition to the parent rock. This soil material is continuously undergoing a change, due to the further influence of weathering and to the gradual accumulation of residues from plant growth. These plant residues besides containing all of the mineral elements essential to plant life, are also the first source for the introduction of organic nitrogen to the soil. As the organic matter and nitrogen resulting from these plant residues increase, the soil becomes better adapted to a greater variety and a more luxuriant growth of plant life. The accumulation of organic matter continues and eventually nitrogen in the soil becomes sufficiently abundant to cause it no longer to be the limiting factor in plant development.

After an adequate supply of nitrogen has accumulated in the soil other factors will tend to limit plant development and of these moisture is by far most important. The influence of moisture in this relationship is so evident that the type and especially the quantity of plant growth produced on soils in nature is in direct proportion to the amount of effective precipitation received. As consistent evidence, the organic matter in arid soil is always more limited than in those soils formed under conditions of heavier precipitation. In the state of Washington, where climatic and soil conditions offer splendid opportunities to study this subject, there is a very direct and close relationship between the amount of organic matter in the soil and the amount of effective precipitation to which such soil has been exposed. Possibly the only reason why this relationship does not hold more true is that the moisture conditions that promote vegetative growth are, as a rule, also those that are very effective in promoting decomposition of organic residues returned to the soil.

Temperature and humidity also have a bearing on organic matter accumulation in that these two factors, if high, play a very effective part in promoting favorable conditions for vegetative growth. However, these same conditions in turn have also an accelerating effect on the rate at which plant residues will decay in the soil. The accumulated organic

matter is therefore the resultant of the total crop residues returned to the soil and the portion decomposed. The highest percentage of organic matter is consequently not necessarily found in those soils where climatic conditions are such as to promote the largest amount of vegetative growth; nor where their influence is such as to prevent its most rapid decomposition. Soils in the temperate zone, where climatic conditions are intermediate from the standpoint of all these factors are, as a rule, best supplied with organic matter.-

The soil in its virgin state is the resultant of rock weathering and organic matter accumulation extending over long geological periods. When soils are first brought under cultivation all tillage practices are directed toward breaking down organic matter in order that more plant food may be made available thus to support more abundant crop yields. The larger plant growth, resulting from such practices, if returned to the soil in total would no doubt, maintain or even increase the amount of organic matter. Under conditions of practical agriculture, however, where a special effort is made to produce large yields and where all the plant material is never returned, there is little hope of even maintaining the soil organic matter. (It becomes evident, therefore, that the organic matter can not be increased above the amount present in the virgin soil unless there is what would virtually constitute a change in climate. The equivalent of such change can be made possible under arid conditions through the application of irrigation water. It is not inconceivable that the seasonal vegetative growth on arid soils can be increased several thousand fold through the application of irrigation water. For instance, the vegetative material produced by a seven ton crop of alfalfa, or a hundred bushel crop of corn, yields which are not uncommon under irrigation, is quite a contrast to the sparse plant life that grew on such soils before water was supplied. This artificial influence on plant growth should also be very effective in increasing the soil organic matter. That this is sound and consistent with actual experience is borne out by the results obtained from investigations in the irrigated sections of this state. The results show that in the course of only a few years of cropping, the organic matter of many of these arid soils because of irrigation has been increased to the point where the difference is easily measurable by analytical methods.

The popular literature on soil management makes many claims for the value and importance of organic matter. It is credited with improving the physical condition of the soil, with checking erosion, with increasing the plant food supply, especially nitrogen, with influencing weathering, with increasing the water holding capacity, with causing more rapid warming up in the spring, etc. While the degree to which organic matter accomplishes all of these benefits will no doubt always be a matter of controversy, it is, however, generally conceded and thoroughly understood

that there is such a close relationship between soil organic matter and productivity that the relationship has long been used as a basis for determining soil fertility as related to crop yields. The relationship is most indicative under virgin conditions where it has not been disturbed by artificial practices like irrigation, tillage, fertilization, etc.

Because of the immediate beneficial results in crop yields from the destruction of organic matter, this phase of the subject is much more thoroughly understood than that pertaining to maintenance. Whether permanent agriculture is more concerned with maintaining organic matter at an arbitrary content or with the proper utilization of such organic matter as accumulates in the soil as a result of regular cropping systems is an open question. In other words, should agricultural practices strive arbitrarily to increase or maintain soil organic matter so that larger yields may be obtained or should they be so conducted that large yields are the primary consideration, thus taking it for granted that the necessary maintenance of adequate organic matter will naturally follow? The correct answer to this question is governed quite as largely by economic as by scientific relationships. In other words, only such methods are practical and worthy of consideration as will result in most direct and immediate profit.

### THE NITROGEN-CARBON RATIO IN SOILS

One characteristic of all soils that is fundamentally important and has been definitely established by many investigators in various parts of the world, is that both nitrogen and organic carbon content furnish a basis for the determination of soil organic matter.

This is a direct approach to the subject and as a result the following equations are generally accepted:

$$\text{Carbon} \times 1.724 = \text{Organic Matter}$$

or

$$\text{Nitrogen} \times 20 = \text{Organic Matter}$$

From these equations it will follow that:

$$\text{Carbon} \times 1.724 = \text{Nitrogen} \times 20$$

or

$$\frac{\text{Carbon}}{\text{Nitrogen}} = \frac{20}{1.724}$$

$$\text{N} : \text{C} :: 1 : 11.4$$

This ratio between nitrogen and organic carbon so well established on experimental data presupposes that there are factors operating in the soil that tend to maintain this ratio in spite of variations in climate, soil type or field practice.

Under virgin conditions it matters little whether the soil be a sandy



loam from an arid section or a clay loam from conditions of heavy precipitation, there is always about one pound of nitrogen for every ten or twelve pounds of carbon. These conclusions are supported by numerous investigators.

•The decomposition of soil organic matter is mainly dependent upon the activities of microorganisms which are composed of protein, and which in common with all materials of this kind have a comparatively narrow nitrogen-carbon ratio. Plant residues as returned to the soil, either in nature or artificially, furnish the main source of energy and food for these organisms and under variable soil conditions their activity is frequently the determining factor in crop production.

•The nitrogen-carbon ratio of plant residues varies from about 1 to 80 in straw to about 1 to 20 in legume materials, while a pure protein product comparable in composition to these microorganisms frequently has a ratio of less than 1 to 10. In order that these microorganisms may carry on their life processes it becomes necessary that plant residues be broken down and utilized. This breaking down process tends to continue until every portion of plant residue will at some time have been very definitely associated with, and will have played a very active part in the growth and reproduction of these organisms. If the ultimate point were reached where all crop residues turned to the soil had been utilized at some time by microorganisms, then the nitrogen carbon ratio of the soil organic matter would be the same as that of the protein material composing the microorganisms. This is the narrowest ratio at which nitrogen and carbon could exist in the soil, and therefore the ratio of nitrogen and carbon in soil organic matter becomes comparatively stable as decomposition causes it to approach this point.\* This narrow ratio is rarely, if ever reached, in virgin soils because there is a continuous return of plant residues having a wider ratio and also because soil conditions are rarely optimum for decomposition for any considerable period. Should these conditions be reversed, however, as is commonly done by farm practice where little effort is made to return crop residues to the soil and where optimum conditions for the decomposition of organic matter are commonly provided, this nitrogen-carbon ratio can be caused to become narrower quite rapidly and thus approach the theoretical ultimate.

Those agricultural practices essential in crop production have a very direct effect on the nitrogen and carbon content of the soil. Cropped soils in all cases not only contain less nitrogen and carbon than the virgin, but the carbon is lost more rapidly than the nitrogen, thus resulting in a narrower nitrogen-carbon ratio.

This narrower nitrogen-carbon ratio is not confined to cropped soils alone but is also found in subsoils. Here too as in cropped soils the ratio becomes narrower as the nitrogen and carbon content decrease and the organic matter is present in lesser amounts.

\* The larger amount of organic matter in the surface soil is easily explained on the basis that it is in this soil volume that by far the most roots are found and besides all crop residues returned are naturally incorporated here. Also under virgin conditions the vegetation has a tendency to draw so heavily on the moisture that during a considerable portion of the growing season the surface soil is generally too dry to meet the requirements for organic matter decomposition. It is only under the artificial conditions of farm practices where special efforts are made to supply the moisture optimum for bacterial activity that organic matter in the surface soil is lost at a rapid rate.

In the subsoil, on the other hand, due to the lesser aeration, conditions for organic matter decomposition may never be optimum at any particular time, but because of the better moisture supply in the subsoil these conditions for decomposition may nevertheless be very favorable for comparatively long periods. This in itself would to some extent at least, explain the narrower nitrogen-carbon ratio in the subsoil, but there is also the matter of leaching which plays an important part.

• When soil organic matter has undergone decomposition to the point where it is about ready to pass into the form of its ultimate decomposition products, a considerable portion assumes a colloidal or soluble state. When in such colloidal or soluble state there is a tendency for it to be leached into the subsoil, its deposition depending upon the amount of precipitation and the soil reaction.\* In arid sections where there is little or no leaching into the drainage systems and where organic matter readily reaches the soluble or colloidal state, because of the generally alkaline reaction of the soil, considerable amounts of organic matter are deposited in the subsoil at depths varying with the penetration of capillary moisture. Wherever such organic matter is deposited it has a narrowing effect on the nitrogen-carbon ratio.

In humid sections where the soils have an acid reaction, practically all of the organic matter is precipitated and held in the surface soil. Here only small amounts of organic matter leach into the subsoil and these are in a more advanced stage of decomposition than the organic matter found in the subsoil of arid sections. The acid reaction not only accounts for the small amount of organic matter in these subsoils, but also for its even narrower nitrogen-carbon ratio.

\* Within the limits of the comparative constancy of the nitrogen-carbon ratio there are variations, however, that are closely associated with the nitrogen content of the soil. There is a tendency for the ratio to become wider with higher nitrogen content. These variations in soil nitrogen content occur in nature as a result of differences in soil texture and in climate as previously discussed.

## MAINTENANCE OF ORGANIC MATTER

Because of the value of soil organic matter in its various relationships and of the fact that organic matter is returned to the soil in the form of crop residues of various kinds and composition, it becomes of foremost importance to determine the influence of such residues upon the maintenance of this important soil constituent.

The practices that have been relied upon for the maintenance, if not the increase in soil organic matter and nitrogen, are the use of crop residues, green manures, barnyard manure and commercial fertilizers. To what extent these materials will accomplish the desired results, depends primarily upon the changes that they must undergo in the soil because of the important bearing that such changes may have upon profitable crop production.

In the return of crop residues, like straw or stubble, materials composed largely of the stalks of plants and which are therefore low in their nitrogen content, the results have not always been very encouraging. Invariably where these materials were returned there was difficulty experienced in maintaining the immediate yield and also there was no evidence that the organic matter had been increased sufficiently to bring about the anticipated improvement in soil conditions.

In determining the merits of materials of this type in their relation to practical agriculture, a mass of interesting data bearing on the decomposition of organic matter has been accumulated.

Many of the practices recommended and followed because of their supposed beneficial effects on organic matter maintenance would find little support if they had to be justified on the basis of actual differences in composition produced in the soil as determined by laboratory methods. The fact, however, that differences in the soil resulting from such practices have not been found by analytical methods employed should not be interpreted as indicating that differences do not exist. We may well question not only our methods for determining influences but also what is actually essential in causing influence. In practical agriculture it has long been recognized that certain treatments or crop sequences have had very important effects on both tilth and yield for which the chemist has been unable to give a satisfactory explanation. To illustrate, the growing of a legume like red clover will not only increase the yield of the succeeding crop but it will also improve the tilth of the soil in a very pronounced degree. These results have generally been attributed to a greater amount of organic matter and nitrogen accumulated. Attempts to justify such conclusions on the basis of chemical analysis, however, have been unsuccessful because the amounts dealt with were rarely sufficiently large to be measurable by that means.

## THE RETURN OF CROP RESIDUES

From the standpoint of permanent benefit to soils the value of the return of crop residues is generally recognized, but frequently the immediate results obtained are not of such a nature as to lend much encouragement to the practice. It is evident that all residues do not have the same effect and in order to encourage their return to the soil the basis for such differences must be understood.

• It has been shown that where the residue, straw, was applied to a large number of different soils, there was an increase in the carbon dioxide evolved and a decrease in the nitrate nitrogen. The depressing effect of straw on nitrate accumulation was gradually overcome as there was more time allowed for decomposition as measured by the total amount of carbon dioxide evolved and nitrate accumulated. This other fact is in evidence, however, that even when the time for decomposition and the amount of straw applied were the same there was still a variation in carbon dioxide evolution and nitrate accumulation which indicates that soil composition may be a prominent factor in the process.

Where several soils of varying nitrogen contents were treated with straw applications there was a depressing effect on nitrate nitrogen accumulation in all cases irrespective of the nitrogen content of the soil and this depressing effect was about equal for all soils. Consequently soils which because of their high nitrogen accumulated nitrates most rapidly still were adequately provided with nitrates after the depressing influence of straw was deducted.

On the basis of the influence of variations in soil nitrogen on straw decomposition it is also reasonable to assume that the amount of straw applied to soil at any one time may be a factor, and the depressing effect of straw applications on nitrate nitrogen accumulation was most prolonged where the applications were heaviest and also this effect was practically in proportion to the amount of straw applied.

• The logical conclusion from these findings is that it becomes increasingly more difficult to utilize straw and strawy manure on soils as such soils become more depleted in nitrogen and organic matter. From this standpoint, therefore, the soil organic matter problem is more easily solved if consideration in this regard is not delayed until soils show evidences of depletion. The return of such crop residues as straw becomes increasingly more difficult as soils reach the stage where they are in greatest need of such treatment.

The fact that straw has a depressing effect on nitrate nitrogen accumulation, and thus develops a condition temporarily unsatisfactory for crop growth, should encourage interest in practices whereby such effect may be overcome. It would seem that one means of control might be to vary the nitrogen content of the residue returned.

\*Where there is an increase in the nitrogen content of the residue there is also a corresponding increase in the nitrate nitrogen accumulation. This is true for carbon dioxide evolution, however, only during the early stages of decomposition. In the later stages the higher nitrogen containing residues cause a lesser evolution of carbon dioxide. The fact that the lower organic carbon losses are associated with the residues of higher nitrogen content is an indication that such losses may be avoided by increasing the nitrogen content of residues like straw. This fact has a fundamental bearing on all those farm practices dealing with soil organic matter maintenance.\*

In attempts to maintain soil organic matter it is not at all uncommon to plow under the entire crop as green manure. Immediate results from such practices as indicated by crop yields, are not always encouraging and in the light of the preceding discussion it is reasonable to suppose that the nitrogen content of green manures which varies with maturity may be largely responsible for the lack of uniform results.

For instance, wheat at an age of 20 days after emergence and when still in the tillering stage contained about four times the nitrogen percentage as was found for that same crop at maturity. Here again the same relationship between the nitrogen content of the residue and nitrate nitrogen accumulation exists. The tendency is for nitrates to accumulate at a lower rate with increased maturity and consequent lower nitrogen content of crop and from this standpoint, there is not much difference between mature wheat and straw. The influence of nitrogen content on decomposition has an important bearing on the practice of plowing under green manures where immediate benefits are desired.

\*The amount of nitrate accumulation is directly influenced by the nitrogen content of the residue. It would appear, therefore, that nitrate accumulation can be fairly closely regulated on the basis of a knowledge of the nitrogen and carbon content of the residue returned.\* For instance, if the rate and amount of nitrate accumulation for straw alone is not sufficient to meet the requirements, this residue may be permitted either to undergo some decomposition before it is applied or it may be supplemented with another material of higher nitrogen content. If on the other hand, the rate and amount of nitrate accumulation from a legume residue is too pronounced for best results, this condition can be controlled by supplementing such residue with a material low in nitrogen.

\*When straw is required to decompose in order that favorable conditions for nitrate accumulation may be established there must necessarily be an elimination of carbon until the desired ratio between nitrogen and carbon is reached in the resulting product. This desirable composition is obtained at the expense of a decided loss in total organic matter. This loss can be overcome and is unnecessary if by some means the nitrogen content of the straw can be increased by treatment.\*

Much of the carbon or organic matter in a low nitrogen residue can be conserved in the process of decomposition if the nitrogen content of such residue is artificially increased by adding nitrogen in either an organic or inorganic form. In cases where strawy manure or crop residues like straw which have a low nitrogen and high carbon content are composted before they are applied to the soil, there is necessarily a heavy loss of organic matter as carbon dioxide. Due to the desirable influence on the physical condition of the soil of all organic matter, irrespective of its composition, it may be of economic importance to conserve this carbon as organic matter. Such conservation may be brought about by adding a nitrogen fertilizer to the high carbon residue thus fixing the carbon in an organic form and thereby increasing the amount of manure produced. Under conditions where organic matter of high carbon content is plowed under as in the case of straw and heavy cereal stubble, a treatment with a nitrogen fertilizer before plowing will not only overcome, in large part, the depressing influence on nitrate accumulation, but will also conserve much of the organic matter contained in the residue, and thus increase its beneficial influence on the physical condition of soil.

### THE PLACE OF SOIL ORGANIC MATTER IN PRACTICE

The history of agriculture teaches that the first consideration in the acceptance of any practice is based very directly on immediate profit. Recommendations to promote the maintenance of yields and the permanence of soil productivity must take this factor into consideration. Unfortunately, all agriculture during its pioneering stage is too frequently profitable only at the expense of soil depletion. On some of the lighter soils which are primarily not over-abundantly supplied with plant food, this system of depletion quickly reaches a point where profitable crop production is no longer possible through further exhaustion and where the cost of reclamation may be prohibitive. The reason the cost of such reclamation becomes high on any soil is that the fertility has declined to the stage where many crops that would have had a beneficial effect on the soil and could have been established without difficulty at an earlier time can no longer be grown without the necessity of special soil treatment or expensive fertilization.

The very life of the agricultural industry necessitates an immediate profit but there is no question but what this same profit could in many cases have been obtained by methods less exhaustive to soil fertility if some of the plant food relationships had been better interpreted, and some of the past exhaustive practices are no longer excusable.

Information on this subject from all sources if properly interpreted leads to just one fundamental conclusion. This conclusion is that the soil organic matter fluctuates with the soil nitrogen and that it cannot be materially increased nor decreased unless soil nitrogen is similarly

influenced. From a fertility standpoint, high crop production to be permanent, requires practices that maintain the soil nitrogen and such practices will then also automatically maintain the soil organic matter.

Among the materials that have been used in the past and are still given foremost consideration in attempts to maintain soil organic matter under field conditions are stubble from cereals or grasses, cereal straws, animal manures, legume stubble, green manure and commercial fertilizers. Although all of these materials, with the exception of the last named, have one thing in common in that they are composed largely of organic matter, nevertheless, it is generally recognized that they are quite different in their influence on productivity. In this discussion it is attempted to show that the nitrogen content of the organic materials applied is a deciding factor in their desirability or value and it is from the standpoint of this nitrogen content that their merits in field practice or practical agriculture should be determined.

Under conditions of straight grain farming where no particular attempt is made to maintain soil fertility or organic matter there is nevertheless a certain amount of roots and stubble returned to the soil. It is conceivable that these residues may contain as much carbon in an organic form as was lost from the soil as carbon dioxide in the process of promoting the decomposition and nitrification so necessary to make adequate plant food available. However, all results from soil analyses made for the purpose of showing the influence of this type of farming on soil composition prove conclusively that there is a gradual decline in the organic matter content. The reason for such decline is explained on the basis that straight grain farming tends to decrease the soil nitrogen content through crop removal and since there is a pronounced tendency for soils to maintain a constant nitrogen-carbon ratio, organic matter can not be maintained but must decline also, in proportion to the decline in nitrogen. The only direct influence on soil organic matter that the return of such materials can have is very limited, for it must be in proportion to the amount of nitrogen in the residues. When residues high in carbon and low in nitrogen are returned to the soil, they always depress nitrate accumulation which is reflected in a decreased yield. This depressing effect becomes more pronounced as soils become more depleted in nitrogen. When the soil nitrogen becomes lower as a result of continuous cropping there develops a strong incentive to avoid the more evident depressing effect of straw and stubble by burning off these residues before plowing. The incentive to burn is most evident where the land is summer fallowed later or is to be again cropped to a cereal the next year. Where an intertilled crop is grown the year following a grain crop or where the land is summer fallowed early before a cereal is again grown, such burning may not be considered necessary. The moisture conserved in the soil as a result of the tillage required by either an intertilled crop or summer fallow

will promote the more rapid decomposition of the residue and its depressing effect will not be in evidence the next year. The amount of nitrogen lost in the burning of straw though small, offers a further proportional hindrance to organic matter maintenance, and it becomes obvious that there is little hope to maintain the productivity where such burning is practiced.

Stubble and sod from grasses like timothy or blue grass have relatively the same influence as the residues from cereal crops with this difference, however, that their nitrogen content is somewhat higher and consequently their depressing effect on nitrate accumulation is less. Although this depressing effect is not as great it is, however, so evident that it becomes desirable to follow such sod with an intertilled crop instead of small grain.

Where straw is returned to the soil, a practice that is frequently recommended on the theory that it affords a means of maintaining soil organic matter, the results in yield are not always encouraging. Due to its low nitrogen content the depressing effect on nitrate accumulation is similar to that for cereal stubble. The length of time for which such depressing effects will be felt, depends very largely upon the amount of straw applied or the conditions favorable for decomposition during the period that this residue is present in the soil. Where adequate moisture for bacterial activity is provided during the warm portion of the year, either as a result of direct precipitation or through conservation by soil mulching practices, decomposition will proceed rapidly and the return to the soil of larger amounts of straw can be recommended. Where moisture conditions are inadequate for decomposition, either straw should not be returned or otherwise should be supplemented with an amount of nitrogen sufficient to overcome the depressing effect. That this can be done and is practical under field conditions has been found.

\* If soil organisms have the power to utilize the nitrogen in an inorganic fertilizer like nitrate of soda to carry on the decomposition of straw it is not unreasonable to assume that the same or similar organisms will avail themselves of some of the atmospheric nitrogen to carry on this process. In other words, the application to the soil of low nitrogen or high carbon containing organic materials may stimulate the activity of free nitrogen fixing organisms. Such activity may result in the fixation of enough nitrogen from the atmosphere to play a part similar to a nitrogen fertilizer in preventing the loss of carbon as previously discussed and thus in part maintaining the soil organic matter supply.

Where animal manures are to be applied to the soil, their value from the standpoint of organic matter maintenance will depend directly upon their nitrogen content. In this regard there is practically no difference between straw or manure, except that the nitrogen content of the latter will vary from that of practically pure straw at 0.5 per cent to about 2.5 per cent depending upon many factors, chief of which is the amount of



composition of the litter used. Manures containing large amounts of litter in the form of straw, shavings, sawdust, leaves or other low nitrogen residues will produce a product of low nitrogen content. Such manure will have practically the same influence on the soil as straw and should be handled in much the same way to avoid depressing effects on such crops as require a ready supply of nitrate-nitrogen. On this basis, manures of low nitrogen content should not be applied either as a surface dressing or plowed under where immediate results are anticipated.

All manures at best contain only small amounts of nitrogen and even when used regularly in liberal applications, they rarely supply more of this plant food element than is removed by the crops grown. As a result of this lack of accumulation of nitrogen, there can be no accumulation of organic matter and consequently in nearly all cases where manure has been used to increase or even maintain soil organic matter, the results have been discouraging. It is only under conditions where abnormally heavy manure applications have been made that there are any indications of organic matter accumulation. In those cases it can generally be shown that there has been more nitrogen added than the cropping system required.

The beneficial effect of a legume on the succeeding crop has long been recognized to the extent that it furnishes the basis for practically all crop rotation systems. It has been taken for granted that this beneficial effect is due to the improvement in the physical condition of the soil and also to the increased nitrogen supplied by the fixing power of the legume. The importance of the latter claim, however, is still in a controversial state, because under non-irrigated conditions, at least, the amounts of nitrogen involved are generally so small as to be difficult of determination by analytical methods. That there is a difference in the soil, however, as a result of growing these crops, can be shown by measuring the amount of nitrate accumulation over a specific period after the legume has been grown. The amounts of nitrates accumulated are practically in proportion to the length of time that the land has been in a legume and these nitrates are an indirect indication of a proportional amount of organic matter left in the soil by the legume.

\*The large amount of available nitrogen found in soils after legumes have been grown makes it customary to select as a succeeding crop one that can utilize large supplies of this element to advantage. Intertilled crops like corn or potatoes fit in very admirably where climatic conditions are such as to make these crops adapted. Small grains, on the other hand, when grown on land abundantly supplied with available nitrogen, will tend to either lodge or "burn" depending upon whether soil moisture is plentiful or scarce during the growing season. Where, because of climatic and soil conditions, farm management practices are such as to make most intertilled crops poorly adapted, it generally becomes necessary to follow

a legume immediately with small grain, a practice meeting with considerable favor in some of the more arid sections. "One method of preventing "burning" under those conditions is to check the rapid nitrate accumulation. This can be done by applying from one to two tons of straw per acre on the legume sod before it is plowed up."

In the use of green manuring crops for purposes of maintaining organic matter, its nitrogen-carbon ratio plays a very important part, not only in the amount maintained, but in the immediate effect on the available plant food, and thus on the yield of the immediate crop. There is a tendency to increase the organic matter in proportion to the amount of nitrogen fixed from the air, and since legume crops are the only ones credited with this function they should be most effective. This theory is borne out by field experience in that the physical condition of a soil is always improved in a very pronounced degree by using legumes as green manure in a rotation, while this improvement is not as prominent and certainly not as residual with non-legumes. Furthermore, legumes, when used as green manuring crops, do not have the depressing effect on yield so frequently experienced with non-legumes. .

All young plants of the various cereal field crops considered suitable for green manuring are higher in their nitrogen percentage or have a narrower nitrogen-carbon ratio than these same plants when more advanced in maturity. This is especially true of non-legumes and it is mainly for this reason that green manuring crops will decompose more readily and prove more beneficial to the succeeding crop if they are plowed under when they are still immature. The unsatisfactory results frequently obtained from green manuring if the crop used is well advanced in maturity before it is turned under have generally been attributed to a lack of proper moisture conditions for the decomposition of the organic matter. That the more advanced maturity of the crop has made a heavier drain on the soil moisture supply is evident and reasonable, but this alone does not explain the poor crop development immediately following such green manuring practice because their depressing influence is also in evidence when there is apparently adequate moisture to meet plant growth requirements.

In the commercial fertilizer industry claims are frequently made that organic products are more desirable because they will tend to maintain the soil organic matter. When one considers the small amount of organic matter so supplied in comparison to the volume and weight of the soil to which it is applied, it becomes quite evident that its value in this connection must be almost insignificant. That nitrogen fertilizers, whether in organic or in inorganic form do, however, exert an indirect influence on organic matter maintenance is more reasonable. The stable relationship between the nitrogen and carbon in the soil indicates that the maintenance of soil organic matter demands the maintenance of nitrogen. Soil

nitrogen may be maintained through the application of nitrogen fertilizers. It would be difficult if not impossible, to show by direct evidence through analytical methods that the use of such fertilizers will maintain the organic matter. Nevertheless, if through increasing the nitrogen supply of the soil better conditions for plant growth are promoted as is so commonly the case, there will follow a more luxuriant development of crops grown and consequently a greater amount of crop residue will be returned. These crop residues would then directly influence the soil organic matter in proportion to the amount of nitrogen they contain. 'Successful agriculture might well adopt the slogan, "You maintain the nitrogen and you maintain all," so far as soil organic matter is concerned.'

# FERTILITY STUDIES ON SOME IOWA PEAT SOILS

W. H. STEVENSON AND P. E. BROWN

*Iowa State College, U. S. A.*

## INTRODUCTION

Peat soils occur in considerable areas in parts of Iowa, the most extensive developments being found mainly in the northwestern central part of the state in what is known as the Wisconsin Drift Soil Area. There are, however, areas of peat somewhat smaller in size in other sections of the state.

The peat occurring in Iowa is extremely variable in character and especially in depth and many of the areas are small and relatively unimportant while others may cover several hundred acres. The agricultural utilization of peat soils is, therefore, frequently a matter of much importance to farmers.

The peats in Iowa have formed in the usual way, by the accumulation of vegetable matter, consisting of swamp grasses, sedges, rushes, flags, etc., in lakes, ponds and poorly drained areas in which the decomposition processes are largely restricted because of the absence of air. The various plants growing in such wet areas leave residues which gradually accumulate and hence the peat which is formed may be quite variable in thickness, depending upon the extent of such vegetative growth and the time during which plant remains have accumulated. The wide differences in character of plant growth also affects the peat and it might be expected that these deposits would vary considerably in depth and composition and hence in fertility.

The Wisconsin drift is a deposit of *débris* left by the most recent glaciation and it is characterized by an immature topography, the occurrence of many lakes, ponds and poorly drained depressions. In fact the major part of this soil area is poorly drained, because of a naturally inadequate drainage system and because of the heavy impervious sub-soils which are common. Hence the major part of the Iowa peats occur in this soil area.

The peat deposits vary in thickness from a few inches to several feet. They differ materially in character because of variations in origin and they occur in various stages of decomposition. When well-decomposed so that there is no evidence of plant structure remaining, the material is known as muck, a term which is commonly misunderstood. It is defined as soil from the parent rock peat. Many areas of peat are sur-

rounded with muck and occasionally muck areas occur through the peat bed. This leads to a still further variation in the characteristics and fertility of peat areas.

Many analyses have been made of the peat in Iowa and the following figures calculated on the basis of one million pounds of surface soil per acre show the wide variation in composition. The average of all the analyses probably show, however, the average composition.

TABLE 1.—Analyses of peat and subsoil under peat calculated on the basis of one million pounds of soil per acre

Surface soil Sample No.	Total nitrogen	Total phosphorus	Total potassium
1	15,311	1,575	7,800
2	12,100	900	6,400
3	13,900	820	5,800
4	4,400	1,200	7,600
5	15,200	1,200	6,200
6	12,300	560	
7	5,900	500	
8	5,800	600	
9	18,300	1,150	
10	8,100	690	
11		1,030	
12		1,050	
Averages	11,130	934	6,760
Subsoils under peats— Average	10,500	2,700	39,466

These figures indicate the low phosphorus and relatively low potassium content of peats. The high nitrogen content is, of course, characteristic. The clay subsoil which is found underlying practically all Iowa peats is high in phosphorus and potassium as the analyses show. No tests were made for acidity as the Iowa peats are almost always very high in lime, effervescing when tested with acids. In this particular they are different from the peats in adjacent states.

## RESPONSE OF PEAT SOILS TO COMMERCIAL FERTILIZERS

Earlier experiments on some shallow peat areas did not show a response to any commercial fertilizers, but some recent tests have indicated that acid phosphate or muriate of potash or the two fertilizers together might prove profitable for use on peat areas. There is often a large increase in the yield per acre and also a much lower per cent of soft corn.

In the following tables (2 to 7) there appear the results of these tests on peat areas in various parts of Iowa, on the Kelley Field, the Lakota Field, the Algona Field, the West Bend Field, the Burt Field and the Northwood Field.

The results obtained on some of these fields, with fertilizers were quite surprising. On the Kelley Field, the acid phosphate gave a large increase in yield and the per cent of soft corn was very low. The muriate of potash had no effect alone or with the phosphate. On the Lakota Field the phosphate, muriate and the two fertilizers had similar effects on the crop but the acid phosphate alone reduced the soft corn per cent the most. On the Algona Field the results were similar to those at Kelley. On the West Bend Field the results were much the same as at Lakota. On the Burt Field the combination of fertilizers was the most effective and on the Northwood Field the muriate of potash was much more effective than the phosphate and the two fertilizers almost doubled the yield over that given by the muriate alone.

TABLE 2.—*Peat experiment, Story County, Kelley Field, 1926* <sup>a</sup>

Plot No.	Treatment	Yield of corn	Soft corn
		bu.	per cent
1	Check	30.7	59
2	Acid phosphate—200 lb. per A.	50.1	10
3	Muriate of potash—200 lb. per A.	28.8	50
4	Acid phosphate—200 lb. per A. + Muriate of potash—200 lb. per A.	42.5	10

<sup>a</sup> The peat in this field ranges from 24 inches to 4 feet in depth and is surrounded by good soil: hence it has been cultivated more regularly and is decomposed to a considerable extent.

These data certainly indicate quite clearly the beneficial effects of acid phosphate and muriate of potash on corn grown on peat soils. In some cases the phosphate is most effective, in other instances the muriate of potash does just as well, while sometimes the two together give by far the best results.

TABLE 3.—*Peat experiment, Kossuth County, Lakota Field, No. 2* <sup>a</sup>

Plot No.	Treatment	Yield of corn	Soft corn
		bu.	per cent
1	Check	35.0	60
2	Acid phosphate—200 lb. per A.	44.4	5
3	Muriate of potash—200 lb. per A.	40.5	40
4	Acid phosphate—200 lb. per A. + Muriate of potash—200 lb. per A.	48.0	20

<sup>a</sup> This peat is from a few inches to 6 feet in depth. It has been farmed for a number of years and is well decomposed.

TABLE 4.—*Peat experiment, Kossuth County, Algona Field, 1926* <sup>a</sup>

Plot No.	Treatment	Yield of corn	Soft corn
		bu.	per cent
1	Check	32.5	95
2	Acid phosphate—200 lb. per A.	68.7	20
3	Muriate of potash—200 lb. per A.	33.7	95
4	Acid phosphate—200 lb. per A. + Muriate of potash—200 lb. per A.	61.2	20

<sup>a</sup> This peat ranges from 12 inches to 3 feet in depth. It has been farmed for some time and decomposition has occurred to a considerable extent.

TABLE 5.—*Peat experiment, Kossuth County, West Bend Field, 1926* <sup>a</sup>

Plot No.	Treatment	Yield of corn	Soft corn
		bu.	per cent
1	Check	48.0	95
2	Acid phosphate—200 lb. per A.	65.5	40
3	Muriate of potash—200 lb. per A.	65.1	50
4	Acid phosphate—200 lb. per A. + Muriate of potash—200 lb. per A.	68.8	7

<sup>a</sup> The peat in this field is from 18 to 24 inches in depth.

TABLE 6.—*Peat experiment, Kossuth County, Burt Field, 1926* <sup>a</sup>

Plot No.	Treatment	Yield of corn
		bu.
1	Check	33.7
2	Acid phosphate—200 lb. per A.	35.2
3	Muriate of potash—200 lb. per A.	39.1
4	Acid phosphate—200 lb. per A. + Muriate of potash—200 lb. per A.	40.2

<sup>a</sup> This peat is 16 to 20 feet in depth. The corn planted was a late variety and did not mature on any of the plots.

TABLE 7.—*Peat experiment, Worth County, Northwood Field, 1926* <sup>a</sup>

Plot No.	Treatment	Yield of oats
		bu.
1	Check	7.5
2	Acid phosphate—200 lb. per A.	17.5
3	Muriate of potash—200 lb. per A.	47.5
4	Acid phosphate—200 lb. per A. + Muriate of potash—200 lb. per A.	80.0

<sup>a</sup> This peat ranges from 4 to 8 feet in depth.

In handling Iowa peat soils it is recommended first of all that the drainage of the area be accomplished. This is naturally the first treatment and the most important. Then the cropping system should be carefully selected. If possible, the growing of vegetable crops is most desirable, but of course, market conditions play a large part in determining the feasibility of this practice. It is not considered practicable to grow corn on newly drained peat but seeding down to timothy and alsike is recommended with subsequent pasturing to compact the peat and permit its decomposition. After a few years of such treatment the peat may be successfully cropped to general farm crops. The use of fertilizers, such as acid phosphates and muriate of potash is apparently profitable and tests of their value on individual peat areas are recommended. Especially for truck crops, will the use of fertilizers prove worth while.



# THE SOIL TYPE AS A FACTOR IN SOIL FERTILITY STUDIES

P. E. BROWN

*Iowa State College, U. S. A.*

## INTRODUCTION

At different times in the past attention has been called to the importance of carrying out all soil fertility studies on the basis of the individual soil type involved <sup>1</sup> but there seems still to be a tendency to consider the matter of minor importance and too often, if any heed at all is paid to type, it is merely to note that the soil used was a sand, a clay, a loam or a silt loam. In fact some investigators seem to look upon the soil type

TABLE 1.—Carrington Loam \*. Profits or losses from fertilizers on various crops

Crop	Treatment	Average yield bu. or tons	Increase for treatment bu. or tons	Value of increase <sup>b</sup>	Cost of fertilizer <sup>c</sup>	Profit or loss
Corn	Check	44.6				
	M	51.4	6.8	\$4.96		
	ML	53.6	2.2	1.61	\$1.00	+\$0.61
	MLRP	56.0	2.4	1.75	3.75	— 2.00
	MLAP	57.1	3.5	2.56	1.95	+ 0.61
	MLCCF	58.1	4.5	3.29	3.63	— 0.34
Oats	Check	45.9				
	M	52.4	6.5	2.86		
	ML	56.2	3.8	1.67	1.00	+ 0.67
	MLRP	59.7	3.5	1.54	3.75	— 2.21
	MLAP	62.8	6.6	2.90	1.95	+ 0.95
	MLCCF	63.2	7.0	3.08	3.63	— 0.58
Clover	Check	1.28				
	M	1.42	0.14	1.84		
	ML	1.55	0.13	1.70	1.00	+ 0.70
	MLRP	1.98	0.43	5.63	3.75	+ 1.88
	MLAP	2.28	0.73	9.57	1.95	+ 7.62
	MLCCF	2.27	0.72	9.44	3.63	+ 5.81

\* 8 series of plots: 26 crops corn, 20 crops oats, 16 crops clover.

<sup>b</sup> Calculated for 73¢ corn, 44¢ oats and \$13.11 clover (av. price for 1913–1922, Iowa Yearbook Agr.).

<sup>c</sup> Cost of application not included.

<sup>1</sup> Jour. Amer. Soc. of Agron. 14: 198.

separations as now practiced as of no practical value and perhaps even of little real technical interest.

It is unfortunate that so much of the work carried out in the past was, of necessity, conducted on mixtures of soil types. There was no recognition of soil types until recently and while extreme differences in soil as to color and texture were, of course, noted, drainage was taken care of and

TABLE 2.—*Grundy Silt Loam* \*. Profits or losses from fertilizers on various crops

Crop	Treatment	Average yield bu. or tons	Increase for treatment bu. or tons	Value of increase <sup>b</sup>	Cost of fertilizer <sup>c</sup>	Profit or loss
Corn	Check	54.1				
	M	58.8	4.7	\$3.43		
	ML	64.0	3.2	3.80	\$1.00	+\$ 2.80
	MLRP	66.2	2.2	1.61	3.75	— 2.14
	MLAP	68.8	4.8	3.50	1.95	+ 1.55
	MLCCF	67.5	3.5	2.56	3.63	— 1.07
Oats	Check	48.9				
	M	54.3	5.4	2.38		
	ML	57.6	3.3	1.45	1.00	+ 0.45
	MLRP	61.9	4.3	1.89	3.75	— 1.86
	MLAP	67.2	9.6	4.22	1.95	+ 2.27
	MLCCF	64.6	7.0	3.08	3.63	— 0.55
Clover	Check	1.95				
	M	1.99	0.04	0.52		
	ML	2.31	0.32	4.20	1.00	+ 3.20
	MLRP	2.61	0.30	3.93	3.75	+ 01.8
	MLAP	2.88	0.57	7.47	1.95	+ 55.2
	MLOCF	2.95	0.64	8.39	3.63	+ 4.76

\* 7 series of plots: 24 crops corn, 11 crops oats, 16 crops clover.

<sup>b</sup> Calculated for 73¢ corn, 44¢ oats, and \$13.11 clover (av. price 1913-1922, Iowa Yearbook Agr.).

<sup>c</sup> Cost of application not included.

the topography was believed to be important, the early experiments on soils were laid out on land which was conveniently located, or which had been bought, or leased or given to the institution. Undoubtedly in many cases the results secured from soil fertility experiments have been affected fundamentally by differences in the soil types and in some instances probably the conclusions have been entirely wrong.

At the present time, however, with the accumulation of knowledge regarding soil types, which has occurred in recent years, there seems little reason, except of course in long time fertility plots and even in these the soil types may be determined, for not carrying out all experimental work with soils on the basis of soil types. It is not sufficient to follow the plan of the crop investigators and consider that a long narrow plot will offset

soil differences. Changes in the occurrence of soil types do not appear according to any cut and dried plan. Long narrow plots may actually accentuate and often do emphasize soil differences. There are many other ideas regarding soil differences which have affected the carrying out of experiments. But there is no question now but that the determination of the soil type is the first thing required in the planning and laying out of field tests, fertilizer experiments or other kinds of soil tests. While all is not known yet about soil types, still there is sufficient information available to permit of a rather accurate separation of the soil types.

TABLE 3.—Carrington Silt Loam <sup>a</sup>. Profits or losses from fertilizers on various crops

Crop	Treatment	Average yield bu. or tons	Increase for treatment bu. or tons	Value of increase <sup>b</sup>	Cost of fertilizer <sup>c</sup>	Profit or loss
Corn	Check	47.1				
	M	55.9	8.8	\$6.42		
	ML	60.1	4.2	3.07	\$1.00	+\$2.07
	MLRP	64.9	4.8	3.50	3.75	— 0.25
	MLAP	63.4	3.3	2.41	1.95	+ 0.46
	MLCCF	63.7	3.6	2.63	3.63	— 1.00
Oats	Check	46.3				
	M	54.6	8.3	3.65		
	ML	57.5	2.9	1.28	1.00	+ 0.28
	MLRP	67.9	10.4	4.58	3.75	+ 0.83
	MLAP	66.6	9.1	4.00	1.95	+ 2.05
	MLCCF	70.5	13.0	5.72	3.63	+ 2.09
Clover	Check	1.47				
	M	1.67	0.20	2.62		
	ML	1.93	0.26	3.41	1.00	+ 2.41
	MLRP	2.27	0.34	4.46	3.75	+ 0.71
	MLAP	2.44	0.51	6.69	1.95	+ 4.74
	MLCCF	2.31	0.38	4.98	3.63	+ 1.35

<sup>a</sup> 5 series of plots: 17 crops corn, 8 crops oats, 12 crops clover.

<sup>b</sup> Calculated for 73¢ corn, 44¢ oats, \$13.11 clover (av. price for 1913–1922, Iowa Yearbook of Agr.).

<sup>c</sup> Cost of application not included.

## INFLUENCE OF IOWA SOIL TYPES

In some field tests carried out in Iowa by the Agricultural Experiment Station the significance of the soil type factor is apparent in the results which are shown in the following tables. In Tables 1, 2, 3, 4, 5 and 6 are given the increases in crop yields the cost of treatments and the profit or loss from the treatments on the Carrington loam, the Grundy silt loam, the Carrington silt loam, the Grundy silty clay loam, the Webster silty clay loam and the Marion silt loam. These are six of the more extensively

developed soil types in the state and they are all quite distinct in character. The Carrington loam and Carrington silt loam are typical Iowan drift soils, the Grundy silt loam, the Grundy silty clay loam and the Marion silt loam are from the southern Iowa loess soil area and the Webster silty clay loam comes from the Wisconsin drift soil area.

TABLE 4.—*Grundy Silty Clay Loam* <sup>a</sup>. *Profits or losses from fertilizers on various crops*

Crop	Treatment	Average yield bu. or tons	Increase for treatment bu. or tons	Value of increase <sup>b</sup>	Cost of fertilizer <sup>c</sup>	Profit or loss
Corn	Check	52.8				
	M	63.0	10.2	\$7.45		
	ML	63.5	0.5	0.37	\$1.00	— \$0.63
	MLRP	65.9	2.4	1.75	3.75	— 2.00
	MLAP	67.7	4.2	3.07	1.95	+ 1.12
	MLCCF	65.6	2.1	1.53	3.63	— 2.10
Oats	Check	47.2				
	M	51.0	3.8	1.67		
	ML	51.7	0.7	0.31	1.00	— 0.69
	MLRP	57.0	5.3	2.33	3.75	— 1.42
	MLAP	57.2	5.5	2.42	1.95	+ 0.47
	MLCCF	58.5	6.8	2.99	3.63	— 0.64
Clover	Check	1.86				
	M	2.51	0.65	8.52		
	ML	2.28			1.00	— 1.00
	MLRP	2.68	0.40	5.24	3.75	+ 1.49
	MLAP	2.85	0.57	7.47	1.95	+ 5.52
	MLCCF	2.71	0.43	5.63	3.63	+ 2.00

<sup>a</sup> 2 series of plots: 8 crops corn, 4 crops oats, 2 crops clover.

<sup>b</sup> Calculated for 73¢ corn, 44¢ oats and \$13.11 clover (av. price for 1913–1922 Iowa Yearbook Agr.).

<sup>c</sup> Cost of application not included.

These types are described as follows:

Carrington loam: dark-brown to black surface soils, subsoils yellow to yellowish-brown or light brown clay; glacial soils; non-calcareous.

Carrington silt loam: similar to loam except for appearance in surface soil texture.

Grundy silt loam: dark brown to black surface soil; a lighter subsurface soil suggesting a gray layer; upper subsoil mottled, heavy plastic; mottling consists of dark drab and yellowish-brown.

Grundy silty clay loam: similar to Grundy silt loam except that texture is heavier; poorly drained naturally.

Marion silt loam: gray white or ash-colored surface soil; Upper subsoils almost white and a silt loam; subsoil gray light yellow to reddish-brown or mottled brownish-yellow, hard impervious clay, poorly drained.

Webster silty clay loam: black surface soil, subsoils mottled gray and brown, heavy in texture ranging from silty clay loams to clays. Subsoils calcareous; of glacial origin level in topography; poorly drained naturally.

TABLE 5.—Webster Silty Clay Loam<sup>a</sup>. Profits or losses from fertilizers on various crops

Crop	Treatment	Average yield bu. or tons	Increase for treatment bu. or tons	Value of increase <sup>b</sup>	Cost of fertilizer <sup>c</sup>	Profit or loss
Corn	Check	56.4				
	M	57.0	0.6	\$0.44		
	MRP	62.7	5.7	4.16	\$3.75	+\$0.41
	MAP	64.8	7.8	5.69	1.95	+ 3.74
	MCCF	65.2	8.2	5.99	3.63	+ 2.36
Oats	Check	50.9				
	M	53.1	2.2	0.97		
	MRP	64.4	11.3	5.72	3.75	+ 1.97
	MAP	67.0	13.9	6.12	1.95	+ 4.17
	MCCF	66.7	13.6	5.98	3.63	+ 2.35
Clover	Check	1.13				
	M	1.19	0.06	0.78		
	MRP	1.33	0.14	1.84	3.75	- 1.91
	MAP	1.63	0.44	5.77	1.95	+ 3.82
	MCCF	1.62	0.43	5.64	3.63	+ 2.01

<sup>a</sup> 2 series plots: 8 crops corn, 4 crops oats, 3 crops clover.

<sup>b</sup> Calculated for 73¢ corn, 44¢ oats, and \$13.11 clover (av. price 1913-1922 Iowa Yearbook Agr.).

<sup>c</sup> Cost of application not included.

The treatments followed in these field tests include manure at the rate of 8 tons per acre once in four years, limestone to neutralize acidity, acid phosphate at the rate of 150 lb. per acre annually, rock phosphate at the rate of 1 ton per acre once in 4 years and a complete commercial fertilizer, a 2-12-2 at the rate of 202 lb. per acre annually in order to supply the same amount of phosphorus as is contained in the acid phosphate.

## DISCUSSION

The average figures showing the total value of the crop increases and the profit or loss on the various soils are given in Tables 7 and 8. Examining these tables it is apparent that there is a wide difference in the response to fertilizer treatments on the different soils. The value of the manure

application has not been calculated but it may be seen from the figures that the total value of the crop increases ranges from \$2.63 on the Webster silty clay loam up to \$34.09 on the Marion silt loam. For lime there is a range from —\$2.95 on the Grundy silty clay loam up to +\$9.25 on the Grundy silt loam. With rock phosphate the results vary from —\$5.96 on the Grundy silt loam up to +\$1.04 on the Carrington silt loam. With acid phosphate, they range from \$7.71 on the Carrington silt loam up to \$15.47 on the Webster silty clay loam. With the complete commercial fertilizer the variation is from —\$2.86 on the Grundy silty clay loam up to +\$12.26 on the Marion silt loam.

TABLE 6.—*Marion Silt Loam* <sup>a</sup>. Profits or losses from fertilizers on various crops

Crop	Treatment	Average yield bu. or tons	Increase for treatment bu. or tons	Value of increase <sup>b</sup>	Cost of fertilizer <sup>c</sup>	Profit or loss
Corn	Check	38.1				
	M	52.8	14.7	\$10.73		
	ML	49.8			\$1.00	—\$1.00
	MLRP	57.0	7.2	5.26	3.75	+ 1.51
	MLAP	55.4	5.6	4.09	1.95	+ 2.14
	MLCCF	58.2	8.4	6.13	3.63	+ 2.50
Oats	Check	35.7				
	M	50.1	14.4	6.34		
	ML	50.2	0.1	0.04	1.00	— 0.96
	MLRP	52.0	1.8	0.79	3.75	— 2.96
	MLAP	58.2	8.0	3.52	1.95	+ 1.57
	MLCCF	62.7	12.5	5.50	3.63	+ 1.87
Clover	Check	1.47				
	M	1.95	0.48	6.29		
	ML	2.13	0.18	2.36	1.00	+ 1.36
	MLRP	2.28	0.15	1.97	3.75	— 1.78
	MLAP	2.74	0.61	8.00	1.95	+ 6.05
	MLCCF	2.82	0.69	9.04	3.63	+ 5.41

<sup>a</sup> 1 series plots: 2 crops corn, 3 crops oats and 3 crops clover.

<sup>b</sup> Calculated for 73¢ corn, 44¢ oats and \$13.11 clover (av. price for 1913–1922 Iowa Yearbook Agr.).

<sup>c</sup> Cost of application not included.

It is evident that the soil type will determine the response to soil treatment and fertilization and the results may vary from a distinct loss from the application up to a large profit from the same treatment on different types. How impossible it becomes, therefore, to make general statements or recommendations regarding the value of soil treatments as applying to all soils. It is a practice to be deplored and it should be definitely understood that all recommendations should be made for a definite soil type or a group of soil types and should not be considered necessarily applicable to

TABLE 7.—*Profits or losses for a four year rotation*

Soil	Treatment	Total value crop increases	Cost of treatment	Profit or loss
Carrington loam	Check			
	M	\$14.75		
	ML	6.59	\$4.00	+\$2.59
	MLRP	10.67	15.00	— 4.33
	MLAP	17.59	7.80	+ 9.79
Grundy silt loam	MLCCF	19.10	14.54	+ 4.56
	Check			
	M	9.76		
	ML	13.25	4.00	+ 9.25
	MLRP	9.04	15.00	— 5.96
Carrington silt loam	MLAP	18.69	7.80	+10.89
	MLCCF	16.59	14.54	— 2.05
	Check			
	M	19.11		
	ML	10.83	4.00	+ 6.83
	MLRP	16.04	15.00	+ 1.04
	MLAP	15.51	7.80	+ 7.71
	MLCCF	15.96	14.54	+ 1.42

other soils. Similarly when investigations of any kind are carried out on soils, the results secured must be applied only to the particular soil type. The sooner everyone comes to a realization of the importance of the soil type factor in soils investigations, the greater will be the value of the work.

TABLE 8.—*Profits or losses for four year rotation*

Soil	Treatment	Total value crop increases	Cost of treatment	Profit or loss
Grundy silty clay loam	Check			
	M	\$25.00		
	ML	1.05	\$4.00	—\$2.95
	MLRP	11.07	15.00	— 3.93
	MLAP	16.03	7.80	+ 8.23
Webster silty clay loam	MLCCF	11.68	14.54	— 2.86
	Check			
	M	2.63		
	MRP	15.88	15.00	+ 0.88
	MAP	23.27	7.80	+15.47
Marion silt loam	MCCF	23.60	14.54	+ 9.06
	Check			
	M	34.09		
	ML	2.40	4.00	— 1.60
	MLRP	13.28	15.00	— 1.72
	MLAP	19.70	7.80	+11.90
	MLCCF	26.80	14.54	+12.26

# THE EFFECT OF SOIL FERTILIZATION ON THE MOISTURE CONTENT, DENSITY, HEAT OF WETTING AND PHOSPHORUS CONTENT OF THE CELL SAP OF PLANTS

M. M. McCool

*Michigan Agricultural Experiment Station, U. S. A.*

## INTRODUCTION

There is available a great mass of data bearing upon the response of crops to fertilizers as measured by yields, but it is evident that there is a paucity of information on the effects of soil fertilization on several plant characteristics, such as the rate of loss of water from tissues, the lowering of the freezing point, the heat of wetting, and the amount of certain mineral elements in the expressed sap and others. We have obtained information on some of these characteristics.

A few years ago when samples of different crops were taken from muck soil fertility experimental fields, it was observed that those grown on the unfertilized plots dried out more quickly than did those produced on the plots that had been fertilized liberally. During the month of August leaves were taken from sugar beets, table beets, Swiss chard and carrots which were growing on the unfertilized plots and plots which had received acid phosphate, and those which had received it and muriate of potash. (It is well to note that acid phosphate when used alone on this muck deposit tends to decrease the yields of the above crops, but when it is applied along with nitrogen and potassium carriers the results are of the reverse order.) They were spread out on a wire screen, so arranged that the upper and lower sides of the leaves were exposed to the air under laboratory conditions. The losses in weight per given weight of material and also unit area were determined by weighing at various time intervals. The results show that on the basis of equal weights the plants grown on the unfertilized plots and on the phosphate treated plots lose weight most rapidly. Whereas the differences are not so striking or so consistent when the unit areas of leaves are compared. It was noted furthermore that the mid ribs required more time to become dry than did the remainder of the leaves. They were larger on the plants grown on the properly fertilized plots than they were on those from the other plots. In addition the results obtained show that in all cases there is more water present in plants grown on the properly fertilized plots than there is in the untreated and phosphate treated plots.



## CONCENTRATION OF THE CELL SAP

It was considered that perhaps the differences in the loss of water from plant tissues was due in part to the differences in concentration of the cell sap. Accordingly the leaves of the above plants were ground immediately after sampling and their freezing point lowerings determined by means of the Beckmann thermometer. The results given in Table 1 show that the applications of potash and phosphoric acid result in appreciable increases in the concentration of the cell sap, but it appears that when the phosphoric acid is used alone it has a tendency to bring about reverse conditions in the case of table beets, and the effects on the sugar beets are slight.

TABLE 1.—*The effect of fertilizers on the concentration of the sap of ground leaves*

Treatment	Sugar beets freezing point lowerings	Table beets freezing point lowerings	Carrots freez- ing point lowerings	Swiss chard freezing point lowerings
None	0.702	0.630	1.118	0.746
K 500	.784	.778		.814
P 500, K 500		.764		
P 750, K 500		.759	1.208	
P 750, K 750	.947	.837	1.258	.834
P 750, K 375		.813	1.238	
P 750	.726	.595	1.190	

## HEAT OF WETTING

The heat of wetting of the finely ground leaves of plants grown on differently fertilized plots was determined because it was considered that this phenomenon is due in the main to colloids. Samples were dried in the oven at a temperature of 96° before the determinations were made. The results obtained show that the heat of wetting of the leaves of plants grown on the fertilized plots is lower than it is on the unfertilized soils; furthermore, they are lower in the earlier stages of the development of several plants than they are in the later stages.

In order to determine the effects of soluble substances upon the heat of wetting, samples of leaves from several plants were leached until the freezing point lowerings were very small. This results in slight increases in the heat of wetting values. This may be the result of the removal of some substances that absorb heat when moistened, as takes place when soils are treated with certain bases.

It would appear from the results obtained that the differences in the rate of drying out of leaves of plants grown on fertilized and unfertilized plots in the main may be due to differences in the concentration of the cell sap, leaf thickness and greater development of the midrib.

## PHOSPHORUS CONTENT OF EXPRESSED SAP

The amount of phosphorus in the expressed juice of several plants as affected by soil fertilization and age of plants has been determined. The experiments reported were conducted in a greenhouse. Usually 2-gallon glazed stoneware jars were used as the containers. Each received 1200 g. either of dry muck or 800 g. of dry mineral soil. In several instances flats 1 foot wide and 2 feet long and  $3\frac{1}{2}$  inches deep were filled with 3000 g. of muck or 20,000 g. of mineral soil. The fertilizers used on the muck were monocalcium phosphate, potassium chloride, and for the mineral soils, urea also. They were added to the soil in solution. The soil was then put through a 4-mesh sieve, mixed, and placed in the containers. The plants were thinned to a uniform stand in all cases.

The treatments shown in the tables indicate pounds of 20 per cent acid phosphate per acre which would be equivalent to the monocalcium phosphate applied. The figures for potassium are in pounds per acre. Urea was used on all mineral soils at the rate of 40 lb. per acre.

TABLE 2.—*The effect of fertilization on the yield and phosphorus content of the cell sap of barley. Period of growth 6 weeks on a muck soil*

Treatment lb. per acre		Yield	Phosphorus in sap
A.P.	KCl		
		grams	per cent
0	500	14.0	0.0030
140	500	24.5	.0101
700	500	41.2	.0190

In obtaining the samples of juice a Watson-Stillman hydraulic press with a  $1\frac{1}{2}$  inch cylinder was used. Immediately after the plant samples were taken they were cut into pieces, ranging in length from 1 to 2 inches, placed in the cylinder and pressure slowly applied. As soon as the pulp

TABLE 3.—*The effect of fertilization on the yield and phosphorus content of the cell sap of barley. Period of growth 5 weeks on a Hillsdale loam*

Treatment lb. per acre		Yield	Phosphorus in sap
A.P.	KCl		
		grams	per cent
0	200	22.1	0.0029
140	200	22.5	.0051
700	200	28.3	.0060

began to appear with the juice, or in case it did not appear, as soon as the gauge indicated 15 tons, the pressure was released. The juice was then centrifuged 5 minutes, poured into a crucible, weighed, dried, 1 cc. of saturated magnesium nitrate solution added, and ignited. The ash was taken up with 5 to 10 cc. of hot 0.2 N HNO<sub>3</sub> diluted to 100 cc. and analyzed. The results are reported on the basis of the weight of juice.

TABLE 4.—*The effect of fertilization on the yield and phosphorus content of the cell sap of barley. Period of growth 13 weeks on a Hillsdale loam*

Treatment lb. per acre		Yield	Phosphorus in sap	
A.P.	KCl		Leaves	Stems
		grams	per cent	
0	500	18.0	0.0020	0.0020
70	500	52.1	.0063	.0104
350	500	68.7	.0360	.0312

According to the data presented in Tables 2, 3, 4, 5 and 6 the phosphorus content of the plant juices increases as the application of monocalcium phosphate to the soil increases. The phosphorus content furthermore is greater in the later stages of development of the plants, that is when grown on soils which have received rather generous amounts of the phosphate, but where the soils are deficient in this element the reverse may be true.

TABLE 5.—*The effect of fertilization on the yield and phosphorus content of the cell sap of beans. Period of growth 8 weeks on a muck soil*

Treatment lb. per acre		Yield	Phosphorus in sap
A.P.	KCl		
		grams	per cent
0	100	17.5	0.0035
70	100	31.5	.0049
140	100	38.5	.0064
420	100	48.0	.0108
840	100	40.8	.0252

Where monocalcium phosphate was applied to soils in amounts ranging from small to large, the maximum growth was obtained where soluble phosphorus could be detected in the soil solution, but an increase in the phosphorus content of the cell sap as well as growth rate was observed where the additions were too small to be detected in the soil solution. The micro-chemical test

employed was improved or modified by C. H. Spurway. The presence of 0.1 p.p.m. of phosphorus in the soil extract can be detected by means of this method.

*TABLE 6.—The effect of fertilization on the yield and phosphorus content of the cell sap of sugar beets. Period of growth 14 weeks on a muck soil*

Treatment lb. per acre		Yield	Phosphorus in sap
A.P.	KCl		
0	500	grams 33.0	per cent 0.0020
140	500	44.5	.0152
700	500	54.1	.0513

# THE INFLUENCE OF MANURE, COMMERCIAL FERTILIZERS, AND LIME ON THE CHEMICAL COMPOSITION OF FIELD SOILS

J. G. LIPMAN, A. W. BLAIR AND A. L. PRINCE  
*New Jersey Agricultural Experiment Station, U. S. A.*

## INTRODUCTION

For centuries it has been the dream of man to enrich cultivated soils by adding to them certain materials or chemicals.

The Romans had learned the value of adding manure and ashes to the soil. Bailey, in his *Cyclopedia of Agriculture* (1) quotes Virgil as follows:

“But sweet vicissitudes of rest and toil,  
Make easy labor and renew the soil,  
Yet sprinkle sordid ashes all around,  
And load with fattening dung the fallow ground.”

For centuries the Chinese have utilized the waste products of human metabolism. In this connection King (5) writes: “Centuries ago China and her sister nations learned, or were compelled, to return to the cultivated fields as nearly as possible the entire volume of human waste from every household, whether in city or country, and statistics obtained through the Bureau of Agriculture, Japan, place the amount of human manure in that country in 1908 at 23,850,295 tons or 1.75 tons per acre of her cultivated land.”

In the early part of the nineteenth century England was using bones extensively as fertilizer. In this connection Hall (4) quotes Liebig: “England is robbing all other countries of their fertility. Already in her eagerness for bones, she has turned up the battle fields of Leipzig, Waterloo and the Crimea. Already from the catacombs of Sicily she has carried away the skeletons of many successive generations. Annually she removes from the shores of other countries to her own, the manurial equivalent of three million and a half of men, whom she takes from us the means of supporting, and squanders down her sewers to the sea. Like a vampire she hangs upon the neck of Europe, nay, of the whole world, and sucks the blood from nations without a thought of justice towards them, without a shadow of lasting advantage to herself.”

About 1800, Washington, in giving instructions for work on his Virginia plantation, wrote (3): “All the hands of the farm not indispensably engaged in the crops, should, so soon as corn planting is completed in the

spring, be uninterruptedly employed in raising mud from the Pocasins and from the bed of the creek into the scow; and the carts, so soon as the manure for the corn and potatoes in 1800 is carried out, are to be incessantly drawing it to the compost heaps in the fields, which are to be manured by it."

In comparatively recent times an enormous commercial fertilizer industry has grown up, and now over a considerable part of the world the products of this industry are being used with the hope of enriching or building up the soil and thus increasing crop yields.

The agricultural investigator at once wants to know to what extent the use of such materials influences the composition of the soil. Is it all used up or dissipated he asks, or is the fertility of the soil actually being increased by the use of these materials? It is therefore perfectly natural that we should look to the soil chemist for an answer to these questions. He is asked to analyze the soil and to give a report as to whether it is gaining or losing in fertility.

Lawes and Gilbert were among the first to carry on extensive investigations along this line. They collected samples of soil from the Rothamsted fields with the most extreme care and made analyses of nine-inch sections to the depth of ninety inches. Without going into a lengthy discussion of their findings suffice it to say that Lawes at least, believed that land under continuous cultivation was being depleted of its fertility. With reference to the general agriculture of Great Britain he said (6): "It is hardly possible to avoid the conclusion that in most cases, profitable agriculture involves slow but continuous exhaustion of the soil."

Although Lawes held this position he recognized full well the difficulty of getting exact information. He says (6): "The evidence brought forward, in regard to fertility and exhaustion enables us to comprehend somewhat more clearly the distinction between the fertility which is part and parcel of the soil, and that which is brought upon the land by the capital or the tenant.

"There is still, however, before those who may be called upon to decide such questions, the very difficult task of ascertaining how much of any imported fertility still remains in the soil, and is available for future crops."

Lawes wrote these words more than forty years ago and the question still remains unsettled. We are still asking the question whether the chemical analysis will show how much fertilizer is needed and whether or not the fertility is being depleted or increased.

The difficulties in the way of giving a concrete answer to these questions are well nigh insurmountable. In the first place the amount of so-called plant food generally applied is so small in comparison with the total amount in the plowed acre, that methods of sampling and analysis now in use can scarcely detect any difference due to an application of fertilizers. To illustrate we may assume an application of nitrate of soda at the rate

of 300 lb. per acre. This would mean about 46.5 lb. of nitrogen for the plowed acre—2,000,000 lb. of soil. This calculated on the percentage basis would be 2 in the third decimal place, which probably means nothing since two samples taken from the treated or untreated plot would differ by as much and perhaps more.

In the humid regions of the United States, fertilizers, where used at all, are generally used in amounts varying from 200 or 300 lb. to more than a ton per acre, the amount depending upon the crop to be grown, the type of soil, distance from market, rainfall, etc. In point of acreage the area that gets the small application far exceeds the area that gets the large application. It is therefore easy to understand that in the great majority of cases it is well nigh impossible, by a chemical analysis of the soil, to measure the influence of applied fertilizers unless the work is carried over a period of years.

The work is further complicated by several forces which are acting on the soil simultaneously. There is the influence of added salts on the soluble and insoluble soil particles; the effect of percolating waters charged with carbon dioxide and carrying soluble salts; the influence of the growing crops on soluble salts, and the effect on soil reaction of crop residues and mineral and organic substances that may have been applied to the soil. There are also temperature changes and the influence of microorganisms and animal life existing in the soil. All these tend to complicate the problem of getting at the chemical changes which are taking place in the soil.

On the other hand, there is no question but that the added fertilizers do bring about important changes in both the chemical and physical composition of the soil. Where specific fertilizer materials or manure or lime are used on the same land over a period of years, it is possible to show by chemical analysis that definite changes have taken place. In another paper (2) this problem has been discussed in connection with cylinder soils.

## INFLUENCE OF FERTILIZERS, LIME AND MANURE UPON FIELD SOILS

### SERIES 1

The field experiments on the availability of nitrogenous materials which have been in progress at this station since 1908 have offered an unusual opportunity for the study of the influence of fertilizers, lime and manure on the composition of field soil.

The soil is a loam inclining to the sandy phase and originally contained about 0.112 per cent nitrogen, 0.10 per cent phosphoric acid and 1.00 to 1.25 per cent potash. The rotation followed on these plots for the first 5 years was a year of corn, 2 years of oats, a year of wheat and a year of timothy. Since that time oats has been grown for 1 year only and

timothy for 2 years. It will be noted that no legume crops are included in this rotation. The plan provides for two sections of 20 plots each having like nitrogen treatments but differing in the matter of lime treatment. The 20 plots designated as section A receive no lime while the 20 plots designated as section B receive lime in the carbonate form at the rate of 2 tons per acre at intervals of 5 years.

A careful record has been kept of the materials added and of the crops removed. The soils have been analyzed at intervals of about 5 years. Table 1 indicates the various fertilizer treatments and also gives the results of the analysis of samples of soil collected at the end of 15 years.

A study of this table brings out some interesting points. Considering first the unlimed section, the A series, it will be noted that there are some rather distinct differences in the percentage of nitrogen. The first 4 plots which have been without nitrogen from the beginning show but little change. One would expect that with 15 years of continuous cropping without nitrogenous fertilizer of any kind, to find the nitrogen content of the soil considerably depleted. It is possible that the location of these plots in the field has something to do with this matter. They are so situated that they receive a slight amount of wash from ground that is somewhat higher.

The percentage of nitrogen in Plots 5 and 6 has been distinctly increased. These plots have received manure at the rate of 16 tons per acre annually since 1908. This is equivalent to approximately 160 lb. of nitrogen per acre annually. This is much more than the average taken out by the crops that have been grown. The amount used, however, is far more than anyone engaged in general farming could afford to use. In other words, on these plots nitrogen has been used in amounts far in excess of what is profitable and they stand as a good illustration of Russell's statement (8) to the effect that the nitrogen supply of cultivated soils is often maintained only at a wasteful expenditure of nitrogenous fertilizers.

Plot 18 receives the same manure treatment as Plot 5 and in addition nitrate of soda equivalent to about 50 lb. of nitrogen, that is, including manure and nitrate of soda, the application is equivalent to more than 200 lb. of nitrogen per acre. It will be noted that the nitrogen content of this plot also has been distinctly increased. Just why Plot 7 has been so much more depleted of its nitrogen than Plots 1 to 4 is not quite clear, though as previously intimated it is probable that the location of Plots 1 to 4 may account for the difference. It will be noted that Plots 9 to 15 inclusive agree quite closely in nitrogen content. These plots receive equal amounts of nitrogen—about 50 lb. per acre annually—but in different forms. The average is a little below the average nitrogen content of the original soils. They have, however, changed but little in nitrogen content during the past 10 years.



TABLE 1.—Analyses of soils from soil fertility plots (Experiment started 1908; samples collected 1922)

Plot No.	Annual fertilizer treatment—1 20 acre plots	Nitrogen (N)				Phosphoric acid (P <sub>2</sub> O <sub>5</sub> )				Potash (K <sub>2</sub> O)	
		Topsoil <sup>c</sup>		Subsoil <sup>c</sup>		Topsoil		Subsoil		Topsoil	
		Unlimed	Limed	Unlimed	Limed	Unlimed	Limed	Unlimed	Limed	Unlimed	Limed
1A, <sup>a</sup> 1B	Nothing	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
2A, 2B	15 lb. muriate of potash	0.102	0.072	0.068	0.051	0.105	0.082	0.082	0.077	1.364	1.038
3A, 3B	32 lb. acid phosphate	.111	.078	.080	.052	.112	.083	.083	.073	1.371	1.030
4A, 4B	Minerals only <sup>b</sup>	.113	.072	.079	.049	.129	.085	.085	.073	1.464	0.984
5A, 5B	Do 1600 lb. cow manure	.134	.128	.085	.092	.160	.143	.101	.120	1.426	1.031
6A, 6B	Do 1600 lb. horse manure	.137	.134	.097	.086	.187	.194	.122	.135	1.186	0.953
7A, 7B	Nothing	.074	.080	.037	.061	.087	.092	.088	.081	1.395	1.228
8A, 8B	Minerals, 8 lb. NaNO <sub>3</sub>	.084	.079	.047	.053	.144	.142	.098	.086	0.742	1.085
9A, 9B	Do 16 lb. NaNO <sub>3</sub>	.097	.074	.064	.052	.135	.120	.099	.079	1.310	1.186
10A, 10B	Do Ca(NO <sub>3</sub> ) <sub>2</sub> equiv. to 16 lb. NaNO <sub>3</sub>	.092	.081	.066	.051	.133	.126	.103	.078	1.267	1.015
11A, 11B	Do (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> equiv. to 16 lb. NaNO <sub>3</sub>	.095	.078	.061	.060	.138	.133	.101	.091	1.353	1.046
12A, 12B	Do CaCN <sub>2</sub> equiv. to 16 lb. NaNO <sub>3</sub>	.086	.080	.048	.056	.125	.126	.073	.072	1.189	1.038
13A, 13B	Do dried blood equiv. to 16 lb. NaNO <sub>3</sub>	.107	.078	.069	.048	.133	.121	.074	.070	1.216	1.104
14A, 14B	Do fish equiv. to 16 lb. NaNO <sub>3</sub>	.108	.081	.072	.060	.176	.159	.098	.090	1.453	1.036
15A, 15B	Do conc. tankage equiv. to 16 lb. NaNO <sub>3</sub>	.087	.075	.055	.061	.165	.159	.088	.103	1.650	1.100
16A, 16B	Do 200 lb. alfalfa hay	.098	.096	.064	.066	.147	.147	.086	.086	1.034	.938
17A, 17B	Do 200 lb. rye straw	.096	.100	.059	.070	.146	.149	.090	.083	1.062	1.050
18A, 18B	Do 1600 lb. cow manure and 16 lb. NaNO <sub>3</sub>	.132	.124	.074	.065	.173	.168	.103	.087	1.445	1.356
19A, 19B	Do only	.091	.082	.059	.050	.135	.134	.079	.065	1.085	1.227
20A, 20B	Do 200 lb. rye straw and 16 lb. NaNO <sub>3</sub>	.094	.091	.060	.056	.140	.137	.085	.070	1.135	1.093
Average		.102	.088	.065	.060	.145	.137	.092	.089	1.216	1.220

<sup>a</sup> A's No lime; B's Limed.<sup>b</sup> Minerals = 32 lb. acid phosphate and 16 lb. muriate.<sup>c</sup> Top soil, 0' 6 3/4"; subsoil, 6 3/4" 13".

Plot 19, without any applied nitrogen, shows practically the same percentage of nitrogen as Plot 20, which receives annually nitrate of soda equivalent to about 50 lb. of nitrogen, and in addition rye straw at the rate of 2 tons per acre. In this connection it must be mentioned that somewhat larger crops have been taken from Plot 20 than from Plot 19.

Without exception there is less nitrogen in the subsoil than in the top soil. It may here be pointed out that the manure used on Plots 5, 6 and 18 has distinctly influenced the nitrogen content of the subsoil of these plots. On the other hand the low percentage of nitrogen in the top soil of Plot 7 is reflected in a very low percentage in the subsoil of this plot. Plot 8 which receives a small application of nitrate of soda also shows a low percentage of nitrogen in the subsoil.

All of these soils are acid having a lime requirement of above 1000 lb. (CaO) per acre. Likewise none of them shows a pH above 6.0.

With only two exceptions the soils of the limed section show a lower percentage of nitrogen than those of the unlimed section. This would seem to bear out the idea expressed by many writers that lime hastens the destruction of organic matter and in this way causes the loss of nitrogen. In the majority of cases the percentage of nitrogen in the subsoils from the limed section is slightly less than in the subsoils of the corresponding unlimed plots. Plot 7 in this section is an outstanding exception.

As would be expected the soils of the limed section are much less acid than those of the unlimed section, nearly all showing a pH of 6.8 to 7.0.

In the majority of cases—both limed and unlimed plots—the percentage of phosphoric acid has been distinctly increased. In one case it has been doubled and in two or three others almost doubled. This supports very nicely the results obtained in cylinder experiments to which reference has already been made. The lime treatment has influenced the percentage of phosphoric acid only slightly, the average for the limed plots being slightly less than the average for the unlimed plots.

The figures for the subsoil of Plots 5 and 6 limed and unlimed, indicate that the manure treatment has also influenced the phosphoric acid content of the subsoils. Plot 18, however, seems to be an exception to this.

All the plots in this experiment have received annual applications of potash except Plots 1, 3 and 7, but according to the analysis Plot 3 now contains 1.464 per cent potash which is above the general average, and indeed stands next to the highest of the entire series. While there is considerable variation in the potash content of these soils, it would appear that this variation is not a result of the potash treatment.

With only slight exception the plots of the limed series show a lower percentage of potash than those of the unlimed series. This is in line with the popular belief that lime liberates potash. Certainly the difference between the two series is wide enough to indicate a greater loss of potash from the limed than from the unlimed series. The difference

between the crop yields in the two series is not great enough to account for the difference in potash content. The same relationship holds in the case of the subsoils insofar as determinations have been made.\*

### SERIES 2

Analyses of samples of soil from another series of plots that have been under cultivation for the same length of time as those above described are reported in Table 2. On these plots a 5-year rotation consisting of three grain crops (corn, oats, wheat) and 2 years of timothy and clover<sup>1</sup> has been carried out.

No farm manure has been used on these plots but commercial fertilizers have been used annually as follows: 300 to 400 lb. of superphosphate, 100 to 200 lb. muriate of potash and a nitrogenous fertilizer equivalent to 160 to 200 lb. of nitrate of soda, per acre. Limestone has been used at intervals of 5 years as indicated in the table under lime treatment.

Analyses made in 1909 show that this soil originally contained 0.112 per cent nitrogen, 0.102 per cent  $P_2O_5$ , 1.00 per cent potash and 1.2 per cent organic carbon. The lime requirement (Veitch method) (9) of the original soil was approximately 1200 to 1500 lb. per acre.

A study of the table shows that the nitrogen content of the soil as determined in 1922 is close to 0.10 per cent; just slightly below the amount in the original soil. It is significant that during a period of 15 years under a system of farming that at the time the work was done would be considered good practice, the nitrogen content of the soil decreased slightly. It is only another verification of Russell's (8) statement to the effect that when plowing and cultivating begin losses of nitrogen set in.

It is fair to state, however, that the amount of nitrogen added in the fertilizer would scarcely replace that taken out by the crop. It is true, there should have been some gain from the clover, but the gain from this source was distinctly reduced by the failure of the crop in 1911 and by rather poor crops in 1916 and 1921. The acid condition of soil of certain of the plots was of course partly responsible for the poor crops of clover. There was a certain equalizing effect here, however, since a poor crop would remove less nitrogen.

An examination of the column giving the percentage of phosphoric acid shows that there has been a slight gain in this constituent. However, with only 300 to 400 lb. of superphosphate to the acre no great gain could be expected, even if we assume that there was very little loss through leaching.

There has been practically no change in the potash content of the soil from the plots in the first group. The samples from the second group of plots show a higher potash content and it would seem that here there has

<sup>1</sup> On a second section of these plots a year of potatoes was substituted for grain during the first two rotation periods.

been a gain. It is quite likely, however, that the difference is to be accounted for through a difference in the location of the plots. Unfortunately potash determinations of the original soil of the plots in this section are not available.

\* The figures for lime and magnesia are not significant. There are slight variations but it cannot be said that either the lime or the magnesia which have been applied have distinctly influenced the percentage of these constituents in the soil. In one case at least the amount in the soil of the check plot is as great as that in the treated plot.

The lime treatment has had a very distinct influence on the soil reaction. This is shown by crop yields previously reported (7) as well as by the determinations of lime requirement and pH shown in Table 2.

TABLE 2.—Analyses of soils from field plots; lime experiments  
(Samples collected 1922)

Lime treatment lb. per acre	Plot No.	Nitrogen		Phos. acid (P <sub>2</sub> O <sub>5</sub> )	Potash (K <sub>2</sub> O)	Lime (CaO)	Mag- nesia (MgO)	Lime Req. Veitch method	Reac- tion
		Soil	Subsoil						
		per cent	per cent	per cent	per cent	per cent	per cent		pH
Check	21	0.082	0.059	0.105	1.023	0.449	0.387	1500	5.2
Calcium limestone	1000	.082	.052	.099	.961	.393	.326	800	6.3
	2000	.084	.051	.100	1.000	.428	.344	400	6.4
	4000	.092	.061	.104	1.046	.505	.348	Alk.	6.8
Magnesian limestone	1000	.095	.062	.107		.400	.337	1000	5.7
	2000	.108	.074	.113		.435	.391	600	6.4
	4000	.108	.071	.108		.576	.514	Alk.	7.0
Check	28	.102	.067	.122	1.410	.548	.549	1400	5.6
Calcium limestone	1000	.101	.065	.120	1.352	.590	.476	900	5.6
	2000	.097	.059	.122	1.278	.590	.507	300	6.4
	4000	.101	.058	.124	1.240	.632	.580	100	6.8
Magnesian limestone	1000	.100	.058	.121	1.329	.569	.529	600	6.4
	2000	.097	.054	.109	1.476	.576	.500	300	6.7
	4000	.100	.049	.101		.612	.536	100	6.9

### SERIES 3

A limited number of analyses have been made on a heavy Sassafras loam soil where potatoes have been grown under fertilizer treatment for a period of 10 years. This soil is peculiarly adapted to potatoes and under normal conditions gives yields of 200 to 350 bu. per acre.

After harvesting the potatoes in this experiment it has been customary to seed a non-legume cover crop, generally wheat (clover was grown one year).

Analyses of samples of soil taken in 1917, when the work was started, show the following: Nitrogen, 0.14 per cent; phosphoric acid, 0.17 per cent and potash, 1.45 per cent.

Analyses made on samples collected from certain of these plots, in the fall of 1926, gave results as indicated in Table 3.

In explanation of the fertilizer treatment it may be explained that Plot 7 has received annually during the 10 years 2800 lb. of a fertilizer analyzing 4 per cent nitrogen, 8 per cent phosphoric acid and 3 per cent potash. Plots 8 and 9 have received the same fertilizer at the rate of 1600 lb. per acre, though they differ in that the nitrogen for Plot 8 is taken from nitrate of soda and that for Plot 9 from ammonium sulfate. Plots 1, 10, 21, and 41 are check plots, that is, they have received no fertilizer during the 10-year period.

A study of the figures shows clearly that the nitrogen supply of the soil has not been fully maintained. Whereas in 1917 it was 0.14 per cent, it is now less than this in all cases, even where the application of fertilizer has been heaviest. In content of nitrogen the check plots have not suffered a serious depletion as compared with the treated plots. Indeed one of them, Plot 21, shows a higher percentage of nitrogen and potash than any of the fertilized plots. (This is probably to be accounted for on the basis of lack of uniformity in the soil.) It is a splendid illustration of the ability of the soil to produce crops for a long time without showing a serious depletion of the fertilizer constituents. At the same time the crops have been drawing upon the reserve plant food materials and another 10 years would show a more serious break.

The phosphoric acid content of the heavily fertilized plot has been increased by 50 per cent and that of the other fertilized plots by about 12 per cent. This is a further confirmation of results secured in cylinder experiments (2) and goes to show that where phosphates are used liberally as in the growing of potatoes the phosphate content of the soil is being increased rather than decreased.

The check plots show a slight decrease in content of phosphoric acid. Although the difference in percentage of phosphoric acid seems small the difference in yield between this plot and the highly fertilized plot is over 100 bushels of potatoes per acre. This undoubtedly means that the proportion of readily available phosphoric acid to total phosphoric acid is far greater in the fertilized plots than in the check plots.

The percentage of potash in the soil from the highly fertilized plot is essentially the same as in the original soil. The remaining plots, with the exception of Plot 41, show slightly higher percentages. The 2800 lb. of fertilizer would supply annually, 84 lb. of potash per acre. This is about the amount that would be required by the average crop removed from this plot (acre basis). The other two fertilized plots have received 48 lb. of potash annually, and the crops taken off have removed considerably more than this. The average crop on the check plots (154 bu. per acre) would remove something over 50 lb. potash per acre annually or a total of a little over 500 lb. for the 10 years. This, however, is a small fraction of the 29,000 lb. that was originally present in the plowed acre. It is quite possible that slight variations in the depth of sampling might account for

the difference noted between the potash content of the original soil and the samples collected in 1926. At least it seems safe to conclude that the potash that was applied over a period of 10 years has not materially influenced the potash content of the soil.

Some interest attaches to the pH determinations. They are all low due to the fact that on these plots no lime has been used during the 10 years. A distinct difference is noted between the pH of Plots 8 and 9. It will be remembered that Plot 8 receives its nitrogen in the form of nitrate of soda while Plot 9 receives its nitrogen in the form of ammonium sulfate. The results confirm the results of other work where these two materials have been used in comparison.

In connection with the figures on the composition of the soil it will be of interest to consider the average yield of potatoes in bushels per acre from the plots that have been referred to. These yields are shown in the last column of Table 3. It may be explained that the averages are made

TABLE 3.—Analyses of soils from fertilized and unfertilized plots—Potato experiment, 1917–1926

Plot	Fertilizer treatment (per acre)	Nitrogen		Phosphoric acid		Potash	pH		Yield pota- toes per acre 8-yr. av.
		Surface soil *	Subsoil	Surface soil	Subsoil	Surface soil	Surface soil	Subsoil	
		per cent	per cent	per cent	per cent	per cent			bu.
7	2800 lb. 4-8-3 <sup>b</sup>	0.123	0.050	0.256	0.079	1.46	4.99	5.89	268
8	1600 lb. 4-8-3	.111	.053	.192	.094	1.56	5.87	6.40	243
9	1600 lb. 4-8-3	.106	.039	.191	.075	1.60	4.80	5.76	201
1	Check (no fertilizer)	.106		.138		1.67	5.42		154
10		.109	.046	.146	.080	1.59	5.35	5.80	
21		.127	.074	.164	.090	1.82	5.36	5.48	
41		.088		.118		1.32	4.99		

\* Surface soil; surface to depth of 3-4 in.; subsoil 8-12 in.

<sup>b</sup> 4-8-3 means 4 per cent N, 8 per cent  $P_2O_5$  and 3 per cent  $K_2O$ .

for 8 years instead of 10, for the reason that the yields for 1923 and 1925 were unusually low and therefore are not considered representative. The check plots have given an average yield of slightly over 150 bushels per acre, whereas the plot that receives 2800 lb. of fertilizer per acre annually has given an average yield of 268 bushels per acre. It may be pointed out that in a period of 10 years the potatoes from the check plot would remove a total of approximately 150 lb. of  $P_2O_5$  per acre, whereas the potatoes from Plot 7 (2800 lb. fertilizer per acre) would, during the same time, remove approximately 257 lb.  $P_2O_5$  per acre. But the check plot has received no phosphoric acid during the 10-year period while Plot 7 has received 225 lb. per acre annually or a total of 2250 lb. A large part of the difference has been used in building up the phosphorus reserve in the soil as shown by the analyses.

It may be seriously questioned whether it is necessary to thus increase the reserve supply of phosphorus in the soil. Work on this problem is already under way and a preliminary report will be given in another paper.

### CONCLUSIONS

Under normal cultural and fertilizer treatment the chemical composition of the soil changes slowly. It is therefore useless for the analyst to expect to be able to detect pronounced differences due to the fertilizer and manure treatments unless the treatments have been excessive or the work carried over a period of years.

Under continuous cropping with cultivated crops it is exceedingly difficult to maintain the nitrogen supply of the soil, even though commercial fertilizers be used every year.

Where a considerable amount of phosphate is applied annually the phosphorus content of the soil is gradually raised, even though large crops be taken off.

With a loam soil containing 1 per cent or more of  $K_2O$ , moderate applications of potash have little or no influence on the potash content of the soil. A limed soil loses more potash than one that receives no lime treatment.

Applications of lime, even as much as 1 to 2 tons of the carbonate per acre at intervals of 5 years, have little influence on the total lime content of the soil. On the other hand, such applications do materially change the pH of the soil.

### LITERATURE CITED

- (1) Bailey, L. H. *Cyclopedia of American Agriculture—Farms*, 1: 372.
- (2) Blair, A. W., and Prince, A. L. 1924. Some changes brought about in cylinder soils by long continued crop and fertilizer treatment. *Soil Sci.*, v. 18.
- (3) Brook, W. E. 1919. *The agricultural papers of George Washington*. (The Gorham Press, Boston) p. 112.
- (4) Hall, A. D. 1909. *Fertilizers and manures*. (E. P. Dutton and Co., New York) p. 108.
- (5) King, F. H. 1914. *Soil Management*. (Orange-Judd Co.) p. 288.
- (6) Lawes, Sir J. B. 1881. *Fertility*. Rothamsted Memoirs, v. 5.
- (7) Lipman, J. G., Blair, A. W., McLean, H. C., and Prince, A. L. 1923. A comparison of magnesian and non-magnesian limestone in some 5-year rotations. *Soil Sci.*, v. 15.
- (8) Russell, Sir E. J. 1921. *Soil conditions and plant growth*. 4th edition. (Longmans Green and Co., New York) p. 181 and 184.
- (9) Veitch, F. P. 1920. Report on the lime requirement of soils. *Jour. Assoc. Off. Agr. Chem.* 3: 371.

# EIN BEITRAG ZUR EINWIRKUNG VON SUPERPHOSPHAT UND RHENANIAPHOSPHAT AUF DEN BODEN

L. VON KREYBIG  
*Cserhátsurány, Ungarn*

Seit Jahren schon mache ich die Erfahrung, dass das Superphosphat auf manchen Tonböden selbst dann keine entsprechenden Mehrerträge liefert, wenn diese Böden stärkeren Bedarf für den Nährstoff Phosphorsäure aufweisen. Auf diesen Böden erreichte ich durch Anwendung basischer Phosphatdünger und insbesondere mit dem Rhenaniaphosphat stets höhere Erträge als mit dem saueren Superphosphat.

Ich hatte schon im Jahre 1924 Gelegenheit das Resultat eines diesbezüglichen praktischen in grösserem Massstabe angelegten Versuches bekanntzugeben (*Actes de la IV-eme Conférence internationale de Pedologie, Rom, III, S. 622, 1924*).

Die seither in dieser Richtung bewerkstelligten praktischen Versuchsergebnisse bestätigten diese Erfahrung in vollem Masses.

Aus der Praxis dürfte es genügend bekannt sein, dass es Fälle giebt in welchen die Erträge trotz reichlich vorhandener Nährstoffe, best entsprechenden Witterungsverhältnissen während der Entwicklung der Pflanzen, und auch Fehlen von Pflanzenschädlingen viel zu wünschen übrig lassen—sich also nicht optimal einstellen können.

Diese Beobachtungen zeigen dahin, dass in solchen Fällen irgendwelche schädlichen Einflüsse sich im Boden geltend machen müssen, welche eine normale Entwicklung der Pflanzen unmöglich machen.

Die Arbeiten von Lemmermann, Kappen, Kirste, u. A. erbrachten Beweise, dass in gewissen Fällen die Ursache dieser nicht entsprechenden Erträge in den krankhaften Aciditätsverhältnissen des Bodens zu suchen sind.

Diesbezüglich in Ungarn durch den Leiter des Bodenkundlichen Laboratoriums der Landwirtschaftlichen Kammer Gyor, Dr. v. Csiky angelegten Freilandversuche erbrachten hierfür vollste Bestätigung.

Wenn wir es also mit solchen krankhaften Böden zu tun haben, so können wir ins solange auf keine optimalen Erträge rechnen bis diese schädlichen Einflüsse durch entsprechende Massnahmen beseitigt werden.

Um den Gründen dieser nicht erfreulichen praktischen Beobachtungen näher zu kommen wurden die im Nachstehenden beschriebenen Untersuchungen durchgeführt.



Nachdem den landwirtschaftlich praktischen Verhältnissen Ungarns den Phosphorsäurekünstdüngern die wichtigste Bedeutung zukommt wurden die Versuche mit dem saueren Superphosphat welches einen pH-Wert von 2,08 und dem basischen Rhenaniaphosphat, das einen pH-Wert von 9,95 (in wässriger Suspension gemessen) durchgeführt.

Die Gründe dessen, dass auf manchen Böden, welche unzweifelhaft einen Bedarf für Phosphatkunstdünger aufweisen und durch die Anwendung von Superphosphat keine so hohen Erträge ergeben als mit dem Rhenaniaphosphat müssen unseren bisherigen Erkenntnissen entsprechend, entweder in der Einwirkung dieser Kunstdüngerarten auf die Aziditäten und einer heirdurch erfolgenden Aenderung der biologischen Verhältnisse, oder in chemischen Gründen gesucht werden, welche beide natürlich Hand in Hand gehen, und zu guter Letzt natürlich auch auf die physikalischen Faktoren ihre Einwirkung ausüben.

Nachdem die endgültige Klarstellung dieser Frage ausser weiteren exact durchgeführten Pflanzenbauversuchen unter gleichzeitiger Beobachtung der Veränderungen der biologischen und auch der bakteriologischen Bodenverhältnisse, sowie sehr vieler chemischer Untersuchungen bedarf wollen nachstehend bekanntgegebene Untersuchungsbefunde die natürlich noch einer *eingehenden Erweiterung* bedürfen, vielleicht nur als grundlegend betrachtet werden.

Um in der angegebenen Richtung Daten zu erhalten wurden die Untersuchungen:

1. Bezüglich der Veränderungen der verschiedenen Aziditätswerte,
2. Die Veränderungen des Stickstoffbindungsvermögens und
3. Bezüglich der chemischen Veränderungen durchgeführt.

*Ad 1.*—Die Einwirkung der Kunstdünger auf die Aziditätsverhältnisse des Bodens, wurde meines Wissens nach bisher nur unter der Anwendung von der Praxis entsprechenden Gewichtsverhältnissen studiert. Es wurden meistens im Gewichtsverhältnisse von rund 3 Millionen kg. Bodengewicht zu 300 kg. Kunstdünger Messungen vorgenommen. Dieses Verhältnis entspricht dem Gewichte nach vollkommen der Praxis und den Verhältnissen, welche in Topfversuchen angewendet werden, bei welch letzteren eine vollkommene Vermischung des Kunstdüngers mit der Erde erfolgt.

Die auf Zugrundelegung dieses oder eines ähnlichen Verhältnissen durchgeführten Messungen zeigen, dass dem Superphosphate keine praktisch zu berücksichtigende Aziditätsverschlechterungen beizumessen sind und wurde das Superphosphat auf Grund dieser Ergebnisse zu den physiologisch neutralen Kunstdüngemitteln eingeteilt.

Es kann heute kaum mehr bezweifelt werden, dass die Wirkung—also nicht die Wirkungsfähigkeit—der Kunstdünger in erster Linie von den chemischen, physikalischen und biologischen Eigenschaften *des Bodens selbst als solchen abhängt*.

In der Praxis werden die Kunstdünger entweder durch ausstreuen und darauffolgendes einackern oder eineggen, welches Einmischen in den Boden grösstenteils erst nach längerer Zeit nach dem Ausstreuen erfolgt, oder aber was sich in Ungarn besonders bewährt hat, durch Anwendung von kombinierten Saemaschinen gleichzeitig mit der Saat in der Nähe des Saatgutes untergebracht.

Ein vollkommenes Vermischen mit dem Boden kann also so wie dies bei den Topfversuchen erreicht wird in der Praxis nie erreicht werden, da die ausgestreuten Kunstdüngerpartikelchen stets *Nesterweise* zu liegen kommen werden. Es dürfte auch sonst genügend bekannt sein dass *der Boden nie homogen ist*, sondern stets schon auf ganz kleine Entfernungen sowohl in den Aziditäts- als auch in den chemischen Verhältnissen messbare Unterschiede zeigen kann.

Die Erträge an Pflanzenmasse richten sich eben jederzeit nach der resultierenden sämtlicher auf das biologische Leben des Bodens und auf das Pflanzenwachstum einflussausübenden Faktoren worunter wir den grössten Einfluss auf jene ausüben können, welche mit dem Boden als solchen in Zusammenhänge stehen. In je höheren Masse wir auf die Durchschnittszusammensetzung des Bodens im günstigen Sinne durch die verschiedensten Massnahmen einwirken desto höhere Erträge werden wir erreichen können.

In sehr vielen Fällen kommt es in der Praxis vor, dass der ausgestreute Kunstdünger überhaupt nicht in die Rhysosphäre gelangt, und doch kann oft auch unter diesen Verhältnissen ein Mehrertrag beobachtet werden, für welche Tatsache wir die Erklärung nur in der durch den Kunstdünger bewirkten erhöhten Tätigkeit der Bodenorganismen eine Erklärung annehmen dürften.

Um einen Einblick in diese Verhältnisse zu gewinnen stellte ich sowohl im Freilande als auch im Laboratorium Versuche an wobei die Versuchsanstellung ähnlich den in der Praxis vorkommenden Verhältnissen gewählt wurden.

Die gestellte Aufgabe war also vorläufig jene, Messungsergebnisse zu erhalten ob im Boden die Einwirkung der ausgestreuten Kunstdünger im Verhältnisse von 3 Millionen zu 300 kg. stattfindet.

Zu diesem Zwecke wurden im Freilande zweimal je 4 □m. Bodenfläche je 20 cm. tief umgeschaufelt und scharf eingereicht. Von jeder Parcellen wurden je 4 Muster gehoben von welchen die Nrn. 1-4 für den Versuch im Laboratorium mit Rhenaniaphosphat die Nrn. 5-8 für den Versuch mit Superphosphat Verwendung fanden.

Die Parcellen 1 wurde sodann mit 120 g. Rhenania die Parcellen 2 mit 160 g. Superphosphat möglichst gleichmässig bestreut.

Die angegebenen Gewichtsmengen an Kunstdünger entsprechen 300 kg. Rhenania respektive 400 kg. Superphosphat auf ein Ha. Bodenfläche. Die Ausstreuung erfolgte am 1. November.

Die chemische Analyse eines Durchschnittsmusters von diesem Boden ergab folgendes Ergebnis:

	%
In conc. Salzsäure löslich	16.08
Chem. gebundenes Wasser	3.16
Humus	2.53
Gesamtphosphorsäure	0.21
Lösliche Phosphorsäure n. 'Sigmond	0.048
CaO	0.63
K <sub>2</sub> O	0.30
Na <sub>2</sub> O	0.20
MgO	0.51
SO <sub>3</sub>	0.25
SiO <sub>2</sub>	6.75
Al <sub>2</sub> O <sub>3</sub>	3.11
Fe <sub>2</sub> O <sub>3</sub>	4.03
Gesamtstickstoff	0.09
Karbonate keine	

Abschlammung nach Atterberg:

	%
Ton	29.6
Mehl	50.5
Sand	18.9
Grobsand	1.0

Der Boden ist ein schwerer Tonboden in guter Kultur und reagiert auf Kalk praktisch sehr gut.

Der Freilandversuch wurde bis Mitte Januar der Einwirkung der Witterungsverhältnisse überlassen und es wurde zur Untersuchung der Einwirkung der Kunstdünger auf die Bodenproben 1-8 im Laboratorium geschritten, welche von den Freilandparzellen vor der Kunstdüngung entnommen wurden.

Zu diesem Zwecke wurden alle Bodenproben in Trichter gefüllt, wobei auf möglichst gleichmässige Verteilung das Hauptaugenmerk gerichtet wurde. Jeder Trichter enthielt ca. 300 g. lufttrockenen Boden und wurde durch jeden 300 cc. dest. Wasser durchsickern gelassen, das Sickerwasser aufgefangen und in den selben die pH Werte bestimmt. Hierauf wurde jeder Trichter in welchem die Erdoberfläche überall gleichmässig 95 cm.<sup>2</sup> war mit den der Praxis entsprechenden 0.28 g. Rhenania- und 0.38 g. Superphosphat (Trichter 1-4 mit Rhenania, 5-8 mit Superphosphat) in 20 cc. dest. Wasser aufgeschlämmt, begossen und mit weiteren 280 cm. dest. Wasser nachgewaschen. Die Sickerwasser wurden wieder aufgefangen und in denselben die pH Werte neuerlich bestimmt.

Nach dem vollkommenen Abtropfen der Sickerwasser wurden aus jedem Trichter je drei Bodenproben Schichtenweise derart entnommen, dass jedes Muster ca. 100 Gramm Erde enthielt. Dies wurde derart bewerkstelligt, dass zuerst die oberste ca. 1 cm. dicke Schichte möglichst

gleichmässig sodann die nächste ca. 2 cm. starke und zum Schluss der Form des Trichters entsprechen die letzte 4 cm. starke Schichte abgekratzt wurde.

Die entnommenen Proben wurden bei Zimmertemperatur getrocknet und im luftfeuchten Zustande die Messungen bewerkstelligt.

Sämtliche Messungen wurden gemäss den Vereinbarungen der Bodenkundlichen Besprachungen und Vorschriften in Groningen durchgeführt.

Die Messungsergebnisse befinden sich in der Tabelle 1.

*TABELLE 1.—Messungsergebnisse bei Verwendung von Rhenaniaphosphat bzw. Superphosphat*

Muster		Originalboden			Filtrate				Schichte	Gedüngter Boden			
					vor Kunstdünger		nach Kunstdünger						
		pH H <sub>2</sub> O	pH KCl	Hydro- lit. Acid	Hp H <sub>2</sub> O	pH KCl	pH H <sub>2</sub> O	pH KCl		pH H <sub>2</sub> O	pH KCl	Hydro- lit. Acid	
1	Versuch mit Rhenaniaphosphat	7,32	6,42	cc. 1,8	6,97	6,49	7,71	6,99	cm 0-1	7,54	6,62	cc. 0,9	
									2-4	7,70	6,65	1,4	
									5-8	7,49	6,55	1,6	
		2	7,45	6,50	1,7	7,41	7,25	7,34	6,97	0-1	7,70	6,67	0,6
										2-4	7,70	6,75	1,4
										5-8	7,65	6,48	1,3
		3	7,28	6,39	1,5	7,18	7,04	7,78	7,45	0-1	7,75	6,72	0,6
										2-4	7,82	6,67	1,7
										5-8	7,67	6,65	1,6
4	Versuch mit Superphosphat	7,56	6,40	1,4	7,46	7,05	7,33	6,71	0-1	7,82	6,62	0,6	
									2-4	7,82	6,65	1,2	
									5-8	7,82	6,60	1,4	
		5	7,31	6,29	1,3	7,27	6,81	7,16	6,81	0-1	7,00	6,60	3,2
										2-4	7,49	6,55	1,8
										5-8	7,38	6,67	1,3
		6	7,58	6,22	1,2	7,39	6,90	7,32	7,02	0-1	7,47	6,46	3,2
										2-4	7,56	6,57	2,3
										5-8	7,61	6,57	1,9
7	6,62	6,41	1,5	7,80	7,45	?	?	0-1	7,54	6,57	3,5		
								2-4	7,61	6,69	2,4		
								5-8	7,61	6,65	1,9		
8	7,71	6,27	1,1	7,69	7,12	6,97	6,66	0-1	7,35	6,46	3,6		
								2-4	7,56	6,53	2,0		
								5-8	7,40	6,69	1,5		

Zur Kontrolle der in der Tabelle 1 erhaltenen Werte wurden noch mit zwei anderen Bodenparten welche ebenfalls Karbonattrei waren und beginnende Verarmung an Basen zeigten dieselben Messungen nach ähnlicher Versuchsanordnung unternommen wobei nachstehende in der Tabelle 2 und 3 verzeichneten Werte gefunden wurden. Bei dem Boden nach Tabelle 3 wurden die Messungen aber nur mehr aus der obersten 1 cm. starken Schichte stammenden Probe vergenommen.

Um zu sehen inwieweit die Verschiebung der Aziditätswerte bei Anwendung von Superphosphat stattfinden kann habe ich dies in einem weiteren Versuche einer Untersuchung unterzogen. Bei diesem Versuche wurden in einem 1 m. langen 3.5 cm. weiten Glasrohre 200 g. eines bereits

TABELLE 2.—Messungsergebnisse anderer Bodenart, bei Verwendung von Rhenania-bezw. Superphosphat

Muster		Originalboden			Filtrate				Schichte	Gedüngter Boden								
					vor Kunstdünger		nach Kunstdünger											
		pH H <sub>2</sub> O	pH KCl	Hydro- lit. Acid	pH H <sub>2</sub> O	pH KCl	pH H <sub>2</sub> O	pH KCl		pH H <sub>2</sub> O	pH KCl	Hydro- lit. Acid						
1	Rhenania- phosphat	6,42	4,82	cc.           8,7	6,21	4,73	6,67	5,02	cm.	6,65	5,06	cc.    2,6						
									0-1				6,85	5,01	8,3			
									2-4							6,68	4,77	7,5
									5-8									
0-1	6,59				5,06	2,84												
2-4							6,80	5,03	7,14									
5-8										6,88	4,87	7,71						
0-1													6,30	4,55	9,30			
2-4	6,18				4,45	9,50												
5-8							6,18	4,47	8,20									
0-1										6,16	4,55	10,1						
2-4													6,26	4,43	9,8			
5-8	6,30	4,55	7,7															

starker hydrolitisch sauren Bodens eingeschüttet und auf demselben 25 gramm Superphosphat aufgeschichtet. Hierauf wurden sukzessive 2000 cc. dest. Wasser durch die Superphosphat-Bodensäule durchsickern gelassen, je 500 cc. der Sickerflüssigkeit wurde immer extra aufgefangen und die Messungen wie früher vorgenommen, deren Resultate in der Tabelle 4 angeführt sind.

TABELLE 3.—Messungsergebnisse noch anderer Bodenart, bei Verwendung von Rhenania und Superphosphat. Aus der obersten 1 cm. starken Schichte allein

Muster	Originalboden			Filtrate				Gedüngter Boden		
				vor Kunstdünger		nach Kunstdünger				
	pH H <sub>2</sub> O	pH KCl	Hydro. Acid.	pH H <sub>2</sub> O	pH KCl	pH H <sub>2</sub> O	pH KCl	pH H <sub>2</sub> O	pH KCl	Hydro. Acid.
Mit Rhenania- phosphat	7,38	5,71	cc.	7,30	5,90	7,33	6,43	7,35	6,28	cc.
Mit Super- phosphat			4,1	7,30	5,97	6,23	5,94	6,88	5,87	6,9

Weiters wurde noch eine Versuchreihe angelegt in welcher die Einwirkung der in Tabelle 5 verzeichneten Kunstdüngermengen auf die Aziditätsverhältnisse immer nur in der ersten 1 cm. starken Schichte gemessen wurde.

Schliesslich wurden Mitte Januar die schichtenweisen Muster des Freilandversuches in der Art entnommen, dass jede Schichte in einer Stärke von je einem cm. nacheinanderfolgend abgehoben wurde. Die Messungsergebnisse sind in der Tabelle 6 enthalten.

**TABELLE 4.**—Verschiebung der Aziditätswerte bei Anwendung von Superphosphat

Muster	Originalboden				Filtrate				Gedüngter Boden			
					vor Kunstdüng.		nach Kunstdüng.					
	pH H <sub>2</sub> O	pH KCl	Hydrol. Acid	Aust. Acid	pH H <sub>2</sub> O	pH KCl	pH H <sub>2</sub> O	pH KCl	pH H <sub>2</sub> O	pH KCl	Hydrol. Acid	Aust. Acid
Superphosphat	2,08								nach Auslaugung 5,07 4,55			
1. Schichte 5 cm.	7,30	5,80	4,1	0,0			500 cc. 5,60	500 cc. 4,49	5,31	3,61	26,47	?
2. Schichte 5 cm.							5,65	4,57	5,75	3,38	28,3	3,9
3. Schichte 5 cm.							5,45	4,61	5,75	3,86	14,5	2,2
4. Schichte 5 cm.							5,68	5,28	5,87	4,34	11,7	2,1

Ohne die in allen Tabellen so ziemlich parallel gehenden Messungsergebnisse einer Diskussion zu unterziehen, da ja dies erst noch weiterer chemischer Untersuchungen bedarf, kann festgestellt werden, dass die Veränderungen in den Aziditätswerten am stärksten in den Filtrationswerten der hydrolitischen Azidität zeigen, und diese Veränderungen ausgesprochen und in fast allen Fällen nur in der obersten 1 cm. starken Schichte stärker bemerkbar machen.

Bei Auswertung der erhaltenen Messungsergebnisse muss *unbedingt* berücksichtigt werden, dass dieselben jederzeit und stets nur *Durchschnittswerte* für die zur Messung herangezogenen Massen ergeben.

Wir dürfen die erhaltenen *Durchschnittswerte* praktisch nicht als vollkommen einwandfrei betrachten, weil eben die sich zeigenden Unterschiede in den parallelen Messungsergebnissen darauf hinweisen, *dass die Kunstdünger nicht gleichmässig dem Gewichte nach auf den Boden,*

**TABELLE 5.**—Aziditätsverhältnisse bei verschiedenen Kunstdüngermengen

Muster			Originalboden			Filtrate				Gedüngter Boden			
						vor Kunstdünger		nach Kunstdünger					
			pH H <sub>2</sub> O	pH KCl	Hydro. Acid.	pH H <sub>2</sub> O	pH KCl	pH H <sub>2</sub> O	pH KCl	pH H <sub>2</sub> O	pH KCl	Hydro. Acid.	Aust. Acid.
Auf 300 g. Boden im Trichter aufgeteilt; nur oberste Schichte gemessen.	g.	Rhenania- phosphat	7,04	6,41	cc.	6,95	6,68	7,03	6,83	7,25	6,42	cc.	
	0,3					6,95	6,70	7,00	6,93	7,44	6,63	0,8	
	0,6					7,00	6,77	7,00	6,93	7,67	6,77	0,4	
	1,2					6,97	6,70	6,96	6,93	7,87	6,98	0,2	
	2,4												
	0,4	Super- phosphat				6,88	6,70	6,88	6,47	6,84	6,21	3,1	
	0,8					6,88	6,72	6,61	6,28	6,28	6,65	4,0	
	1,6					6,91	6,68	6,08	5,89	6,31	5,79	12,4	1,8
	3,2					6,91	6,72	5,66	5,55	6,02	5,68	16,0	2,6

TABELLE 6.—Messungsergebnisse verschiedener Schichten bei verschiedener Kunstdüngung

Muster	Muster bis —cm. Tiefe	Werte		
		pH H <sub>2</sub> O	pH KCl	Hydro. Acid.
1. Ohne Düngung	1	6,78	6,16	1,2
	2	7,56	6,20	1,8
	3	7,60	6,14	1,7
2. Do	1	7,87	6,16	1,6
	2	7,84	6,20	1,1
	3	7,82	6,20	0,8
3. Rhenaniaphosphat	1	7,85	6,25	1,0
	2	8,30	6,19	0,9
	3	7,87	6,20	1,3
4. Do	1	7,82	6,37	0,9
	2	8,26	6,49	1,0
	3	8,08	6,35	0,6
5. Superphosphat	1	7,70	6,20	3,1
	2	7,87	6,19	1,9
	3	7,50	6,19	2,9
6. Do	1	6,72	5,90	3,9
	2	6,83	5,83	4,6
	3	7,42	5,90	3,1

also weder im Verhältnisse 100:0.01 noch in solchen wie die Schichtenweise Probenahme ergab, sondern ich möchte es vielleicht am besten mit dem Worte Nesterweise ausdrücken, wirken.

Ich glaube m. E. nur wenig gegen die Objektivität zu verstossen, wenn ich annehme, dass es in den zur Messung herangezogenen Massen gewiss noch kleine Nester geben wird, welche noch eventuel weit grössere Unterschiede in den Aciditäten aufweisen als die erhaltenen Zahlen aufweisen.

Wenn es uns möglich wäre ein solch kleines Nest für sich herauszunehmen und in diesem die Aziditätsverhältnisse zu messen wurden wir meines Erachtens nach sicherlich derartige Unterschiede finden, welche auf eventuel durchwachsende Pflanzenwurzeln und auf die Bodenmikrobentätigkeit fühlbaren Einfluss ausüben können. Dass die Bodenmikroben ihr Leben Nesterweise fristen ist ja bekannt.

Ad. 2. Veränderungen des Stickstoffbindungsvermögens.—Mit den Bodenmustern mit welchen die in der Tabelle 1 und 2 erhaltenen Werte erhalten wurden, wurden weiters Impfversuche nach der Azotobaktermethode Niklas angestellt.

Boden 1 besitzt zwar Azotobakter von Natur aus, dieselben sind aber in sehr wirkungsschwachen Zustände vorhanden. Der Boden zeigt geringen Phosphorsäure und stärkeren Kalkbedarf. Wenn die Azotobaktermethode nach Niklas parallel unter Anwendung von Rhenaniaphosphat und Superphosphat vorgenommen wird, ist die Kahlhautentwicklung bei Anwendung von Rhenaniaphosphat sehr stark bei Superphosphat aber schwächer als bei Anwendung von Kalk ohne Phosphorsäurezusatz. In der Praxis zeigte auf diesem Boden Rhenaniaphosphat grössere Erträge als Superphosphat.

Bei Boden nach Tabelle 2 ist ausgesprochener starker Phosphorsäurebedarf und Kalkbedarf nach der Azotobaktermethode gefunden worden. In diesem Boden war Azotobakter von Natur aus nicht zu finden.

Um einen Einblick zu gewinnen in wie weit die Anwendung der verschiedenen Phosphatkunstdünger und Kalk auf das Stickstoffbindungsvermögen *in diesen beiden Böden* ausüben, wurden mit denselben nachstehende quantitative Impfversuche angestellt.

Es wurden je 4 Kölbchen mit je 6 gramm lufttrockener Erde beschickt und erhielten die ersten 3 Kölbchen je 20 cc. einer Nährlösung welche 2% Mannit, 0,025%  $MgSO_4$ , 0,02% KCl enthielten, die 4-ten Kölbchen erhielten ausserdem in der Nährlösung noch 0,03%  $K_2HPO_4$ . Zu dem Kölbchen 1 wurde auf die 20 cc. Nährlösung noch 0,0266 g. Superphosphat, zu dem Kölbchen 2 zu den 20 cc. Nährlösung noch 0,02 g. Rhenaniaphosphat und zu dem Kölbchen 3 noch 0,6 g.  $CaCO_3$  zugegeben.

Hierauf wurden die Kölbchen alle mit je einer Platinöse einer sehr wirksamen Azotobakterimpferde beimpft welche ich von Herrn Professor Simon erhalten habe für was ich an dieser Stelle nochmals meinen besten Dank ausspreche. Hierauf wurden die Kölbchen 3 Wochen bei 25 Grad bebrütet.

Die Analyse ergab nach dieser Zeit bei den verschiedenen Versuchen nachstehende *Zunahmen* an Stickstoff:

Boden 1:

	mg.
Kölbchen 1 mit Superphosphat, Stickstoffzunahme	14.28
“ 2 “ Rhenaniaphosphat, “	17.96
“ 3 “ Kalk, “	17.40
“ 4 “ norm. Nährlösung, “	16.00

Boden 2:

	mg.
Kölbchen 1 mit Superphosphat, Stickstoffzunahme	12.41
“ 2 “ Rhenaniaphosphat, “	15.19
“ 3 “ Kalk, “	12.67
“ 4 “ norm. Nährlösung, “	13.51

Bei beiden Böden zeigte also das superphosphat geringere Stickstoffzunahmen als das Rhenaniaphosphat.



*Ad. 3. Chemische Veränderungen.*—Es wurden ebenso wie bei der Prüfung der Veränderungen der Aziditätsverhältnisse die Böden in die Trichter gefüllt und der eine Trichter mit 1.9 gramm Super- der andere mit 1.4 gramm Rhenaniaphosphat bestreut. Es wurden bedeutend grössere Mengen an Kunstdünger verwendet als den praktischen Verhältnissen entsprechen da ja Hauptzweck war zu sehen wie die Verteilung der Phosphorsäure im Boden unter Einwirkung von sehr viel Wasser stattfindet. Nach Ausstreuen der Kunstdünger wurde soviel dest. Wasser aufgeschüttet dass die Sickerwasser je 500 cc. betragen haben welche aufgefangen wurden. Die Bodenmenge war in allen Fällen je 300 gramm. Es wirkten also auf 300 g. Boden 500 cc. Wasser welches Verhältnis einer Niederschlagsmenge von ca. 500 mm. in der Praxis entspricht.

In den Sickerwassern- je 500 cc. wurde die Kieselsäure das Aluminium und Eisenoxyd und die Phosphorsäure bestimmt.

Es wurde weiters der Boden wieder Schichtenweise aus den Trichtern entnommen so dass die Stärke der ersten Schichte ca. 1 cm. betrug und aus diesen Schichtenproben die Gesamtposphorsäure nach der conc. Salpetersäuremethode, die lösliche Phosphorsäure nach der Citronensäuremethode Lemmermanns in 1%-iger Citronensäure bestimmt.

In nachstehender Tabelle sind die erhaltenen Resultate verseichnet und bezieht sich wie gesagt die Analysenzahlen der Sickerwasser auf je 500 cc. jene des Gehaltes vom dem Bodenschichten an  $P_2O_5$  auf bei 100 Grad bis Gewichtconstantz getrocknetem Boden.

Der untersuchte Boden entstammte einem Schlage welcher auf Phosphorsäurekunstdüngung nach der Azotobaktermethode Niklas nicht reagierte.

Das bei den Versuchen verwendete Superphosphat war ein seit 4 Jahren bei mir liegendes Knochenmehlsuperphosphat, welches einen Gesamtposphorsäuregehalt von 23.2% und in 1% Citronensäure lösliches 15.84%  $P_2O_5$  hatte.

Das Rhenaniaphosphat hatte einen Gesamtposphorsäuregehalt von 30.02% und einen in 1% Citronensäure löslichen Gehalt von 20.5%; im übrigen war dasselbe nach der Analyse folgend zusammengesetzt:

	%
$SiO_2$	9.23
$Fe_2O_3 + Al_2O_3$	3.01
$CaO$	41.32
$MgO$	0.59
$Na_2O$	14.41
$K_2O$	0.53
$SO_3$	0.68

Ohne aus den erhaltenen Zahlen weitergehende Folgerungen ziehen zu wollen, umsomehr als ja die diesbezüglichen Untersuchungen fort-

gesetzt werden, um eben einen möglichst weitgehenden Einblick zu gewinnen, ist aus denselben unbedingt ersichtlich, dass der ausgestreute Kunstdünger sowohl das wasserlösliche Superphosphat als auch das Rhenaniaphosphat in der obersten 1 cm. Schichte des Bodens festgelegt hat und das Superphosphat wasserunlöslich geworden ist. Es wurde aber aus dem Superphosphate ersichtlich eine grössere Menge so stark festgelegt, dass dieselbe selbst durch 1% Citronensäure nicht mehr gelöst werden konnte.

Die bisherigen Untersuchungsergebnisse zeigen sowohl in den Einwirkungen der geprüften Kunstdünger auf die Aziditätsverhältnisse als auch in der Einwirkung auf das Stickstoffbindungsvermögen und auch in den Untersuchungen betreffs der quantitativen Festlegung der zugeführten Phosphorsäure *weitgehende Übereinstimmung* und kann ohne den weiter im Gange befindlichen Untersuchungsergebnissen vorzugreifen aus denselben schon jetzt gefolgert werden, dass:

1. Die Einwirkung von Super- und Rhenaniaphosphat auf die Böden mit welchen die Versuche durchgeführt wurden nicht auf eine 20 cm. starke Bodenschichte erfolgt sondern sich meist schon in höchstens einer solchen von 1 cm. auswirkt.

2. Die Einwirkung auf die Aziditätsverhältnisse dieser Böden durch die angewendeten Kunstdünger zeigt sich am stärksten in den Veränderungen der Titrationsaziditäten u. zw. nur Schichtenweise.

3. Das Superphosphat hat seine Wasserlöslichkeit vollkommen eingebüsst und wurde sogar die Löslichkeit in 1% Citronensäure gegenüber jener vom Rhenaniaphosphat ungünstig beeinflusst.

4. Es kann ohne gegen die Objektivität zu verstossen aus den verschiedenen Messungsergebnissen gefolgert werden, dass die Einwirkung der in Untersuchung gewesenen Kunstdüngerarten auf den Boden jederzeit nesterweise erfolgt ist.

5. Für die Praxis weisen vorstehende Untersuchungsbefunde, dass die praktische Wirkung auf den Pflanzenertrag welche ja zu guter Letzt Zweck jeder Kunstdüngung ist, weitgehend auch von der Unterbringungsart abhängig ist. Der Phosphatkunstdünger muss eben jederzeit in jene Bodenregionen kommen in welchen die intensivste Tätigkeit der Mikroben stattfindet.

Der Zweck vorstehender Untersuchungen war keineswegs allgemein gültige Regeln aufzustellen und Folgerungen abzuleiten, da ja solche in der praktischen Landwirtschaft wohl ganz ausgeschlossen sind. Ich wollte nur die Aufmerksamkeit darauf lenken dass wir bei der praktischen Beratung vorstehend geschilderte Verhältnisse stets mitberücksichtigen müssen, was ja wohl von sachverständiger Seite stets der Fall sein wird.

Der Vorgang der Diffusion, die Wasserbewegung und die Bodenbearbeitung sorgen unbedingt dafür, dass mit der Zeit die Nesterweisewirkung eine Verteilung findet jedenfalls bestätigen aber vorstehende Unter-

suchungsergebnisse, dass eine Niederschlagsmenge von 500 mm. hierzu kaum beigetragen hat.

Zum Schlusse erübrigt mir noch den Herrn Professor von Sigmond und Kappen meinen aufrichtigen Dank für ihre wertvollen Ratschläge auszusprechen und meinen Assistenten Herrn Ebenspanger Julius für seine Hilfeleistung bei den Durchführung der Untersuchungen zu danken.

# TYPES OF SOIL AND THE PHOSPHATE REQUIREMENT OF POTATOES

A. W. BLAIR AND A. L. PRINCE

*New Jersey Agricultural Experiment Station, U. S. A.*

## INTRODUCTION

The more progressive potato growers of the country have come to believe that a ton of high grade fertilizer to the acre is not too much for potatoes. This usually means about 1000 lb. of 16 per cent superphosphate, supplying 160 lb. of phosphoric acid ( $P_2O_5$ ). It is now a well established fact that phosphoric acid is but slightly lost in the drainage waters, and it is also well known that the potatoes taken from an acre of land remove a comparatively small amount of phosphorus. Analyses have shown that a 200 bushel crop will remove about 19 lb. of phosphoric acid, and since the vines are not taken from the land, it is very evident that under the fertilizer practice mentioned above the land is receiving a large excess of phosphoric acid. What then becomes of all the phosphorus that is applied to the potato fields, in the great potato growing sections of the country, in the course of 25 or 30 years?

The amount of analytical work that has been done on potato soils that have been under continuous cultivation for a period of years is limited, but through some experimental work that will be reported later in this paper, it has been shown that the annual application of 2800 lb. of commercial fertilizer to the acre for potatoes for a period of 10 years has resulted in increasing the phosphoric acid content of the soil by about 50 per cent.

In another experiment (general farm crops) analyses made on a loam soil that has been under a 5 year rotation for 25 years, and where superphosphate was used to give about 100 lb. of phosphoric acid per acre annually, show a gain of about 1000 lb. of phosphoric acid per acre.

In another case where superphosphate and manure were used annually, the percentage of phosphoric acid in the soil was doubled in a period of 15 years.

In view of this accumulation of phosphoric acid in soils where the crops grown remove more phosphoric acid than is removed by a potato crop, it seems worthwhile to ask, first: Is there a need for so much phosphoric acid for potatoes? And, second: Is there a danger of causing injury and thus depressing the yields where so great an excess of phosphoric acid is used?

Results obtained with potatoes on Penn loam soil at this Station, through several years of experimenting with varying amounts of superphosphate, gave some indication of a depressed yield with the heavier applications of phosphate. This work suggested the laying out of an experiment for the purpose of studying the effects of varying amounts of superphosphate and rock phosphate in the growing of potatoes on different types of soil. For this work three soils were chosen. (1) Colts Neck loam having an unusually high percentage of iron and phosphoric acid. (2) A poor Sassafras loam low in nitrogen and phosphoric acid. (3) Portsmouth loam (a poorly drained soil) high in organic matter, total nitrogen and phosphoric acid, and strongly acid in reaction.

Partial analyses of these soils are herewith given:

	Colts Neck loam	Sassafras loam	Portsmouth loam
	per cent	per cent	per cent.
N	0.126	0.053	0.451
P <sub>2</sub> O <sub>5</sub>	0.772	0.108	0.202
K <sub>2</sub> O	1.660	0.950	1.341
CaO	0.660		
MgO	0.750		
Fe <sub>2</sub> O <sub>3</sub>	25.180		
Al <sub>2</sub> O <sub>3</sub>	5.840		
Organic carbon		1.380	5.710
pH	6.5	6.2	5.2
Lime requirement (Veitch)	100	600	4000

The soils were brought to the Experiment Station and after being passed through a screen and thoroughly mixed, were placed in outdoor cylinders which have a surface area of 3 square feet. The depth of the soil in the cylinders was about 10 inches, the subsoil being that which was originally present where the cylinders were located. The cylinders were laid out in three series of 40 each to accommodate the three soils. In the case of each soil nitrogen and potash were applied to all cylinders in generous but uniform amounts, so that a deficiency of these might not become a limiting factor. After half the cylinders in each series were given a liberal application of lime in the carbonate form, the superphosphate and rock phosphate were applied as indicated in Table 1, the superphosphate being applied in amounts of 100, 200, 500 and 1000 lb. per acre and the rock phosphate in amounts giving phosphoric acid equivalent to that in the superphosphate. In each series check cylinders, that is, without phosphate, were provided and all treatments were carried out in duplicate.

The soils were placed in the cylinders in the fall of 1922 and the Sassafras loam and Portsmouth loam were immediately planted to rye with the

fertilizer treatment as described above. The Colts Neck loam was seeded to barley early in the spring of 1923, the fertilizers being used here also in accordance with the plan. The rye and barley were harvested at maturity and the grain and straw saved separately for analysis.

The figures for these crops are not reported here for the reason that the phosphate treatments had no effect upon the yield of dry matter, the averages for the check plots being essentially the same as those for the treated plots. Neither did the treatments influence in any way the percentage of phosphoric acid in the grain and straw.

Following the grain crops soybeans were seeded, without fertilizer treatment, on all of the cylinders and this crop was harvested late in the summer as hay. Here also the phosphate treatment did not at all influence the yield of dry matter, the average for the check plots again being essentially the same as the average for the phosphate treated plots. Neither did the phosphate treatment influence the phosphoric acid content of the soybean hay.

Following the soybeans a winter cover crop of rye was seeded on the Colts Neck and Sassafras soils, and in the spring of 1924 this was turned under in preparation for potatoes. About the middle of April fertilizers were applied to the cylinders in accordance with the plan, and Irish Cobbler seed potatoes planted. In the matter of cultivation, spraying, etc. the treatment was uniform on the three types of soil.

Under the same fertilizer treatment potatoes were again grown in 1925 and in 1926. The 1925 crop was preceded by a winter cover crop of alfalfa and the 1926 crop by a cover crop of rye and vetch. In both years the cover crops made rather poor growth due in part to the caking of ice on the cylinders during the winter.

The yields on the Sassafras loam for 1925 and 1926 were influenced to some extent by a diseased condition of the plants and are so irregular as to make it inadvisable to attempt an average of the three years.

### YIELDS OBTAINED FROM COLTS NECK LOAM

The yields for this soil are shown in Table 1. Considering first the three year average for the limed section, it will be noted that there was some increase over the check when the superphosphate was raised to 200 and 500 lb. per acre. With 1000 lb. per acre the average yield dropped to 411 g. which is only a little more than the average yield on the check cylinder.

With the rock phosphate the average yields are slightly below those with the superphosphate. When the average for the three years is considered the check shows a higher average than any of the treated plots.

On the no-lime section the averages for the superphosphate are slightly less than the averages for the corresponding treatments on the limed section; also as in the no-lime section, the highest yields were obtained

with the 200 and 500 lb. application of superphosphate. The 1000 lb. application gave a yield only a little above that of the check.

The yields with rock phosphate on this section are generally lower than those on the superphosphate section. In this case the highest yields were obtained with the 100 and 200 lb. application. The average yield with the 1000 lb. application was less than with the check.

TABLE 1.—Yield of potatoes with superphosphate and with raw rock phosphate—Soil: Colts Neck loam

Superphosphate lb. per acre	With lime					Without lime				
	Check	100	200	500	1000	Check	100	200	500	1000
Year	grams <sup>a</sup>	grams	grams	grams	grams	grams	grams	grams	grams	grams
1924	446.9	464.3	502.1	430.7	346.1	417.2	458.4	581.3	524.2	501.1
1925	409.3	375.5	384.3	382.5	437.5	314.5	320.0	344.3	359.3	327.3
1926	359.6	375.9	530.5	551.5	449.4	370.4	394.1	438.5	407.5	335.8
Average	405.3	405.2	472.3	454.9	411.0	367.4	390.8	454.7	430.3	388.1
Rock phosphate <sup>b</sup>										
Year										
1924	432.5	372.0	402.8	354.8	421.7	486.6	469.9	494.0	361.6	470.8
1925	369.0	370.5	335.3	271.3	194.8	286.3	394.3	298.8	267.3	276.5
1926	410.0	434.0	410.4	350.4	420.1	402.5	423.4	405.4	318.9	347.0
Average	403.8	392.2	382.8	325.5	345.5	391.8	429.2	399.4	315.9	364.8

<sup>a</sup> Weights are given in grams per cylinder (1/14,520 part of an acre).

<sup>b</sup> The rock phosphate contains  $P_2O_5$  equivalent to the amount used on the corresponding superphosphate cylinder.

Taking the results for the three years it would appear that when used with superphosphate the lime increased the yields slightly. With rock phosphate the difference in yield between the limed and unlimed sections is not appreciable. The yields were slightly larger with superphosphate than with rock phosphate. With superphosphate the 200 and 500 lb. applications gave some increase over the check, but the 1000 lb. application did not give an appreciable increase.

### YIELDS OBTAINED FROM SASSAFRAS LOAM

The yields for this section, for the three years, are shown in Table 2. As previously pointed out the yields for 1925 and 1926 are so out of harmony with those for 1924 that it seemed inadvisable to undertake an average of the three years. Some consideration, however, may be given to the yields for 1924. In this case the difference between the limed and unlimed sections is not great, and since the soil is not strongly acid this is what might have been expected. There is, however, a slight difference in favor of the unlimed section.

Likewise the difference between the superphosphate and rock phosphate

is not great. In the limed section the 1000 lb. application did not give an appreciable increase over the 200 and 500 lb. application.

In the no-lime section the superphosphate shows a slight advantage over the rock phosphate.

TABLE 2.—Yield of potatoes with superphosphate and with raw rock phosphate—Soil: *Sassafras loam*

Superphosphate lb. per acre	With lime					Without lime				
	Check	100	200	500	1000	Check	100	200	500	1000
Year	grams*	grams	grams	grams	grams	grams	grams	grams	grams	grams
1924	501.0	536.2	580.2	628.3	608.6	605.0	672.3	656.1	641.9	775.1
1925	80.0	104.5	94.3	157.8	193.5	100.3	46.3	23.3	143.5	160.3
1926	164.4	234.7	273.7	270.8	441.3	22.6	62.4	60.4	47.7	161.3
Rock phosphate <sup>b</sup>										
Year										
1924	552.6	477.2	625.3	537.7	571.5	581.4	581.9	610.4	597.8	673.5
1925	50.5	77.5	164.0	49.5	57.5	12.5	87.5	79.3	73.5	63.8
1926	260.2	166.7	137.5	90.8	117.1	43.0	35.3		32.7	31.3

\* Weights are given in grams per cylinder (1/14,520 part of an acre).

<sup>b</sup> The rock phosphate contains  $P_2O_5$  equivalent to the amount used on the corresponding superphosphate cylinder.

On account of the partial failure of these crops for 1925 and 1926 the results obtained on this soil may not be considered conclusive, but if we may judge by the yields obtained in 1924, it would seem that the yields obtained with 500 and 1000 lb. of the phosphates are not enough larger than those obtained with the 200 lb. application, to justify the expense of the larger applications.

### YIELDS OBTAINED FROM PORTSMOUTH LOAM

The yields for this soil are reported in Table 3. When the three year averages are studied it is found that in most cases the yields are slightly larger with lime than without lime. Where lime was used there was very little difference between the average yields with superphosphate and rock phosphate. In the case of the check plots and the plots receiving the 1000 lb. application the advantage is in favor of the rock phosphate. In the rock phosphate section the highest average yields were obtained with the 500 and 1000 lb. application.

In 1926, however, the highest yield in this section was obtained on the check plot.

It is interesting to note that on this soil which contains a high percentage of organic matter and which was originally strongly acid, the yields with rock phosphate were quite as good, on an average, as those with the superphosphate. It is a confirmation of the claim that has often been made, namely: that rock phosphate shows a higher availability on



TABLE 3.—Yield of potatoes with superphosphate and with raw rock phosphate—Soil: Portsmouth loam

Superphosphate lb. per acre	With lime					Without lime				
	Check	100	200	500	1000	Check	100	200	500	1000
Year	grams <sup>a</sup>	grams	grams	grams	grams	grams	grams	grams	grams	grams
1924	610.8	689.7	706.7	687.9	672.8	504.3	575.4	571.1	749.6	839.5
1925	251.5	266.5	212.8	283.0	287.0	250.8	125.3	254.3	193.8	202.8
1926	334.4	395.5	367.4	499.8	299.1	264.6	263.4	287.8	407.6	328.1
Average	398.9	450.6	429.0	490.2	419.6	339.9	321.4	371.1	450.3	456.8
Rock phosphate <sup>b</sup>										
Year										
1924	649.0	703.4	600.9	764.6	805.1	674.5	629.6	722.4	715.4	706.9
1925	257.5	261.3	236.5	345.0	377.0	273.3	249.5	170.3	278.3	258.5
1926	447.2	420.1	272.5	323.4	389.1	251.3	304.9	286.6	320.4	248.3
Average	451.2	461.6	370.0	477.7	523.7	399.7	394.7	393.1	438.0	404.6

<sup>a</sup> Weights are given in grams per cylinder (1/14,520 part of an acre).

<sup>b</sup> The rock phosphate contains  $P_2O_5$  equivalent to the amount used on the corresponding superphosphate cylinder.

acid soils and soils high in organic matter than on soils that are more nearly normal.

## FIELD EXPERIMENTS

To supplement the cylinder work on the phosphate requirement of potatoes a field experiment was planned with plots 1/40 acre in size and work started in 1924. The soil is a Sassafra loam of medium quality, and had been in corn several years previous to the potato work.

Twelve plots were provided, with fertilizer treatments as follows:

	N	$P_2O_5$	$K_2O$
	per cent	per cent	per cent
4 check plots, fertilizer analyzing	4	0	4
2 plots, fertilizer analyzing	4	4	4
2 Do	4	8	4
2 Do	4	12	4
2 Do	4	16	4

The fertilizer was applied at the rate of 1600 lb. per acre, being drilled in the row at the time of planting. The potatoes were planted with a 2-horse planter. Irish Cobblers were used in 1924, and Green Mountains in 1925 and 1926. Following the crop of 1924 rye was seeded as a winter green manure crop and following the crop of 1925 rye and vetch were seeded.

The yields for the three years are given in Table 4. For the check treatment the average of 4 plots is given and for all the other treatments the average of 2 plots. Considering only the primes, it is noteworthy that the highest average yields were made on the plots that received the 4-4-4 fertilizer and the next highest on the plots that received the 4-8-4. The yields with the 4-16-4 are very nearly the same as those on the check plots, while those with the 4-12-4 are only a little better.

TABLE 4.—Yield of potatoes with varying amounts of phosphoric acid—Field experiment

Plot No.	Fertilizer treatment			1924		1925		1926	
	N	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O	Primes	Seconds	Primes	Seconds	Primes	Seconds
1, 4	per cent	per cent	per cent	bu.	bu.	bu.	bu.	bu.	bu.
7, 11	4	0	4 (check)	85.9	33.7	33.5	14.8	85.3	22.9
2, 8	4	4	4	106.7	39.2	42.0	12.0	118.3	28.8
3, 9	4	8	4	100.9	47.4	37.5	15.8	113.4	33.0
5, 10	4	12	4	89.9	39.3	26.0	16.5	92.1	30.5
6, 12	4	16	4	82.7	45.5	28.5	15.3	87.9	33.5

### •GENERAL CONCLUSIONS

The question of the proper amount of phosphorus for potatoes is one that cannot be definitely answered until carefully planned experimental work can be carried out on a number of types of soil and over a period of years.

The preliminary work here reported would lead one to doubt the necessity of making heavy applications of phosphate for this crop every year. Certainly there is indication that in some cases at least the results would be quite as satisfactory with 200-500 as with 1000 lb. of superphosphate.

Since potatoes *appear* to require large amounts of phosphoric acid (though the amount they take out of the soil is small), it is possible that one of the functions of superphosphate is that of precipitating toxic substances, as for example, soluble aluminum compounds. If this view is correct then the amount of phosphate to be used could, in many cases, be materially reduced by using a moderate amount of lime, in those sections where the scab fungus is not likely to become a problem..

# EASILY REPLACEABLE CALCIUM IN RELATION TO RETURNS FROM LIMING <sup>1</sup>

F. L. DULEY

*Kansas State Agricultural College, U. S. A.*

## INTRODUCTION

It has been a common practice for experiment station and extension workers to use the results of simple soil acidity tests as the chief basis for recommendations to farmers regarding the need of their soils for lime. This has been due to the fact that acidity has been popularly considered the principal cause for the soil condition found where liming gives increased yields of certain crops. This idea has persisted in spite of the fact that a few workers have pointed out that no close correlation exists between soil acidity and returns from liming when different soils are considered. Many soils that are distinctly acid have been shown to produce good yields of any of the common field crops and do not give a significant return for liming. On the other hand some soils showing only slight acidity give very marked increases from applications of lime.

It is generally admitted that there are a number of factors which will affect the returns that can be obtained from liming. Among these, the most important are, the kind of crop to be grown, the fertility of the soil, the amount of available basic material, and the "soil acidity." The fact that a number of factors are involved would seem to preclude the possibility of using any single test as an absolute criterion of the need for lime.

The question naturally arises as to whether it may not be possible to make determinations other than those of acidity which will represent the need for lime more accurately. In the work reported in this paper soils from experiment and demonstration fields in the seven corn belt states of Missouri, Wisconsin, Kansas, Iowa, Illinois, Kentucky and Ohio, were collected and studied. On these experiment fields the response to liming has been determined by direct field tests. An attempt was made to determine the relation, if any, between the lime needs of these soils as shown by experiment and the amount of calcium that can be extracted by means of 0.04 *N* carbonated water. Some of the results of earlier work have been reported in previous papers (1, 2).

## CHEMICAL METHODS

Twelve grams of soil were shaken with 600 cc. of 0.04 *N* carbonated water for two hours. The samples were then filtered through special suction filters. Duplicate 500 cc. aliquots were then evaporated to dry-

<sup>1</sup> Contribution No. 170, Dept. of Agron., Kansas Agr. Expt. Sta.

ness and the calcium content determined by precipitating as the oxalate and titrating with 0.05 *N*  $\text{KMnO}_4$ .

### METHODS OF COMPUTING RETURNS FROM LIME

In order to compare the returns from lime on as nearly an equivalent basis as possible all results are expressed in value of the crop per acre, considering wheat worth \$1.00 a bushel; oats \$0.40 a bushel; corn \$0.50 a bushel; soybean seed \$2.00 a bushel; clover seed \$10.00 a bushel; soybean and clover hay \$10.00 a ton, and alfalfa hay \$12.00 a ton. The increases for all crops in a four year period or rotation were added together and the cost of liming, assumed to be \$3.50 a ton for ground limestone, was deducted. The results are considered as net returns for liming.

It has been impossible so far to secure data that are entirely satisfactory regarding the returns from lime and considerable allowance for error must be made owing to the fact that the field results have been obtained with different cropping systems and varying amounts and different forms of liming materials. It is believed, however, that the results are sufficiently accurate to enable one to select the soils giving large returns and those giving the lowest returns. In fact, this method should be very nearly in line with the practical results to be expected.

### RESULTS

Table 1 gives the amount of soluble calcium, the net returns for liming, the pH value and the acidity for the soils on 11 experimental fields. The

TABLE 1.—*Relation between soluble calcium and returns from liming on soils showing strong acidity*

Location	Trough test	pH value	Ca per acre soluble in 0.04 <i>N</i> $\text{H}_2\text{CO}_3$			Net returns from lime per 4 yr. rotation
			0-7 in.	7-12 in.	Total 0-12 in.	
Moran, Kans.	st	5.5	357	612	969	\$14.81
Columbus, Kans.	st —	4.9	408	571	979	9.88 <sup>a</sup>
Cuba, Mo.	st	5.6	423	395	818	10.73
Parsons, Kans.	st	4.9	490	347	837	9.12 <sup>a</sup>
Wooster, Ohio	st —	5.7	490	531	1021	9.18
Union, Mo.	st	5.7	510	525	1035	11.12
Davenport, Ill.	st —	5.6	520	792	1312	5.28
St. James, Mo.	st	5.5	521	510	1031	6.68
Vandalia, Mo.	st	5.5	630	515	1145	4.53
Rest, Kans.	st	5.3	688	745	1433	-4.52 <sup>b</sup>
Aledo, Ill.	st —	5.3	945	1665	2610	-1.05

<sup>a</sup> Results calculated for alfalfa only.

<sup>b</sup> Analyses of Kansas soils made by W. H. Metzger, graduate assistant on soils.

results are shown graphically in Fig. 1. These soils all show strong acidity as measured by the Truog or Zinc Sulfide method. The pH values range from 4.9 to 5.7. It will be seen that there is a very wide range in the value of the crop returns. The amount of calcium soluble in 0.04 N  $\text{H}_2\text{CO}_3$  when calculated on the basis of 2,000,000 lb. of soil shows a variation in the surface soil from 357 lb. per acre to 945 lb. per acre. Moreover,

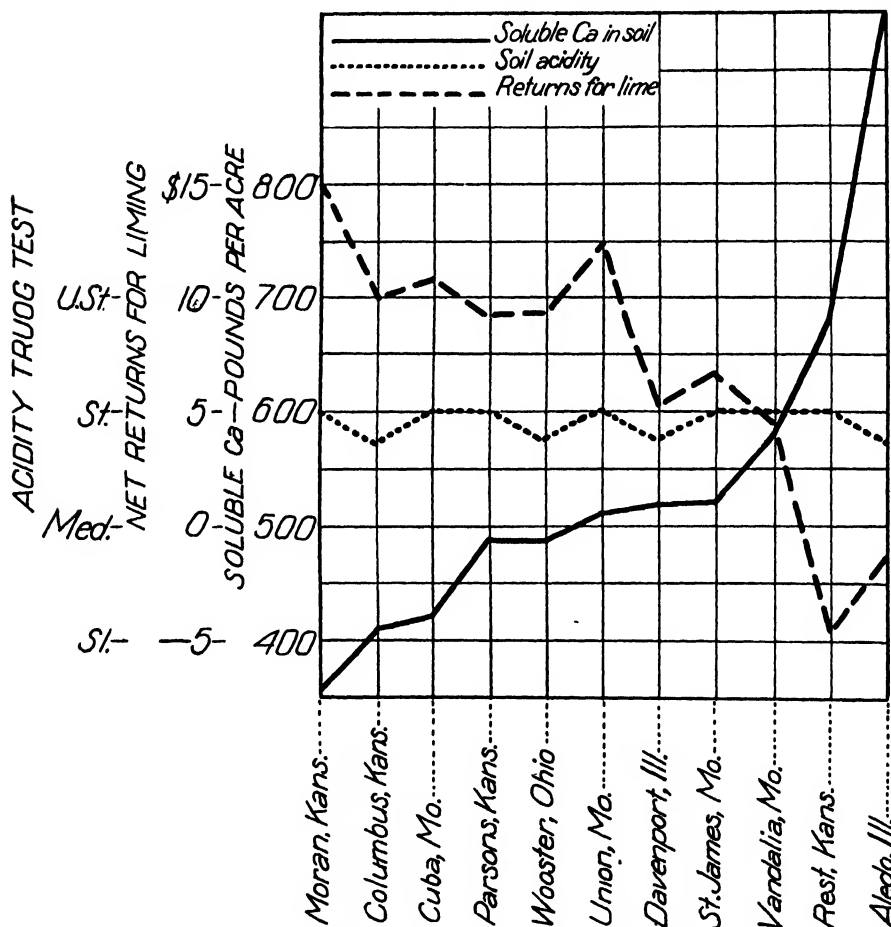


FIGURE 1.—Relation between soluble calcium and returns from liming on soils showing strong acidity

there seems to be a very close inverse relation between the amount of soluble calcium and the net returns. In those cases where the soluble calcium exceeds approximately 700 lb. per acre in the surface soil or 1300 lb. in the surface foot of soil the returns for liming are greatly diminished. Where soils have materially less than these amounts the returns from the use of lime are decidedly higher. This would indicate that from a practical standpoint when soils have less than 700 lb. soluble

calcium per acre in the surface 7 inches a profitable increase from liming under ordinary conditions in the corn belt can usually be expected. For certain crops liming may give profitable increases even where the soluble calcium content is somewhat higher than 700 lb. per acre.

There are certain cases where the soluble calcium content of the subsoil may be radically different from the surface soil. If the subsoil has more calcium as at Moran, Kansas, alfalfa may give less response to lime after it is well established than during the first year or two. It is desirable wherever possible to have the calcium determinations on both the surface and subsoil.

TABLE 2.—*Relation between soluble calcium and returns from liming on soils showing medium acidity*

Location	Truog test	pH value	Ca per acre soluble in 0.04 N H <sub>2</sub> CO <sub>3</sub>			Net returns from lime per 4 yr. rotation
			0-7 in.	7-12 in.	Total 0-12 in.	
El Dorado Springs, Mo.	med	5.3	490	428	918	\$6.28
Strafford, Mo.	med	5.9	511	425	936	7.25
Windsor, Mo.	med -	5.6	570	635	1205	8.66
Manhattan, Kans.	med +	5.8	632			6.60
Balmont Co., Ohio	med -	5.5	705	680	1385	1.58
Maryville, Mo.	med -	5.9	711	855	1566	-0.37
Union Grove, Ill.	med +	5.7	880	615	1495	-0.16
Ft. Scott, Kans.	med	5.3	938	1204	2142	-2.84

Table 2 and Fig. 2 show similar results for soils of medium acidity. In this group as in those of strong acidity the soils having the lower amounts of soluble calcium give decidedly higher returns for liming than the soils having over 700 lb. per acre in the surface 7 inches of soil. As before there is seen to be a wide variation in the soluble calcium content even in

TABLE 3.—*Relation between soluble calcium and returns from liming on soils showing slight acidity*

Location	Truog test	pH value	Ca per acre soluble in 0.04 N H <sub>2</sub> CO <sub>3</sub>			Returns from lime per 4 yr. rotation
			0-7 in.	7-12 in.	Total 0-12 in.	
Greenville, Ky.	sl	5.7	436	395	831	\$3.31
Stark City, Mo.	sl	6.7	530	630	1160	4.04
Willow Springs, Mo.	v. sl	6.7	716	615	1321	1.37
Russellville, Ky.	sl +	6.3	731	773	1504	1.87
Paulding Co., Ohio	sl -	6.5	1370 -	1480 -	2850	-10.66

soils where the acidity remains practically the same. The pH values of these soils seem to check no better with the returns from lime than the results of the zinc sulfide test.

Table 3 gives the results for liming on slightly acid soils. For the few cases available the agreement between the Truog test and returns for

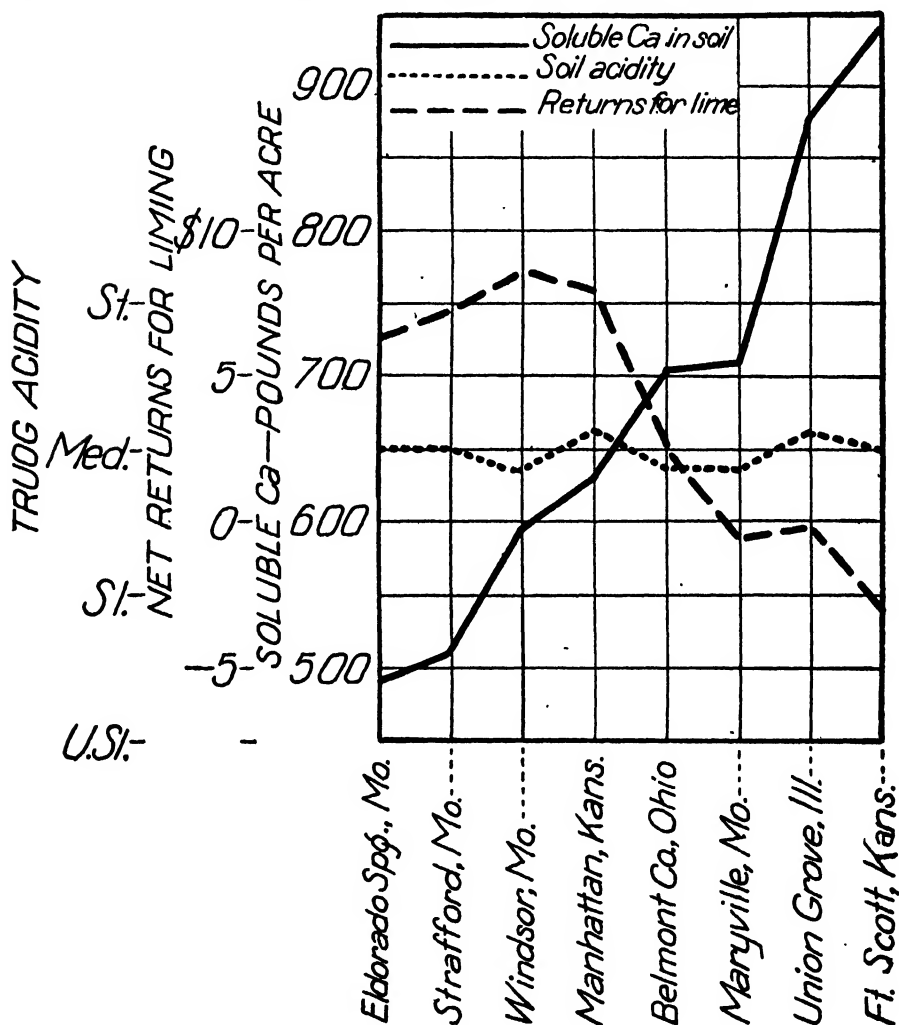


FIGURE 2.—Relation between calcium soluble in 0.04  $\text{NH}_4\text{CO}_3$  in surface soil, and returns for liming on soils showing medium acidity

lime is better than for those cases previously discussed as will be apparent from a comparison of Fig. 3 with Figs. 1 and 2. However, it is a rather striking fact that some of the slightly acid soils have given good returns for liming; in fact much better than some of the more strongly acid soils.

While the average returns for each of the strong, medium, and slight

acidity groups would show some general correlation between returns for lime and soil acidity, there are striking exceptions in each group. This fact seems to greatly diminish the value of soil acidity tests when applied to different soils, for making recommendations for practical applications of lime.

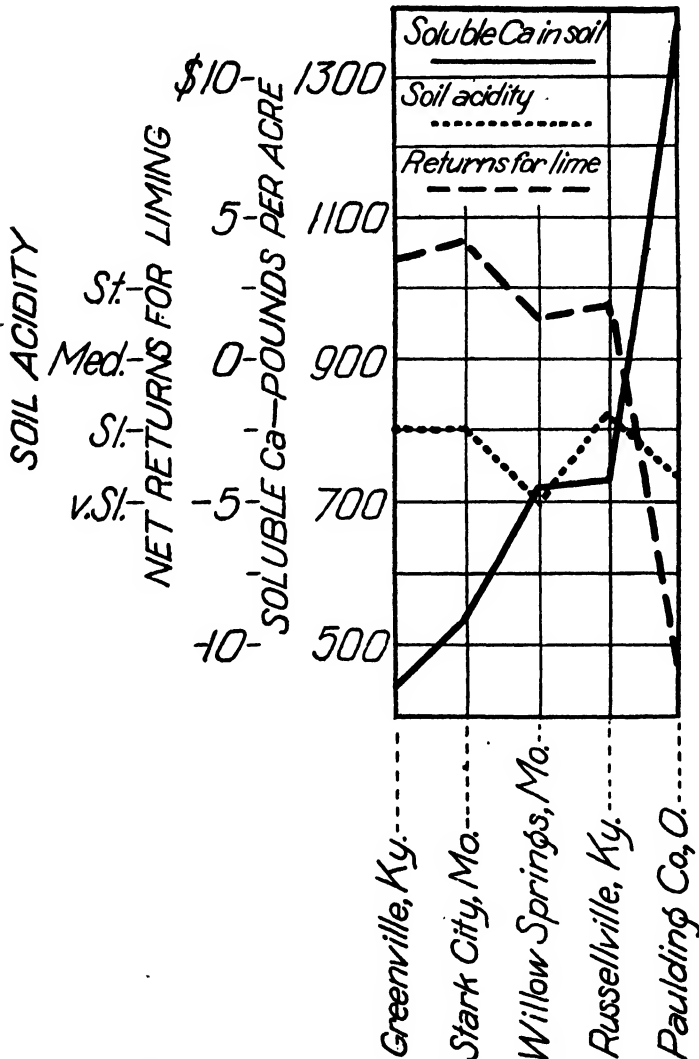


FIGURE 3.—Relation between soluble calcium and returns from liming on soils showing slight acidity

In Table 4 the data on soluble calcium are assembled in relation to acidity. It will be seen that for any group of these soils having approximately the same acidity, there is a wide variation in the amount of replaceable calcium to be found in the surface 7 inch layer.



When certain soils have two or three times as much easily soluble calcium as others, it does not seem reasonable to assume that the same results can be expected from the addition of liming materials. The results of field tests seem to justify this conclusion.

TABLE 4.—*Variation in soluble calcium in soils having approximately the same acidity*

Number of soils reported	Acidity Truog test	Range in soluble Ca; pounds per acre
11	strong	357 to 945
8	medium	490 to 938
5	slight	436 to 1370

In Fig. 4 will be found curves showing the results from all soils herein reported arranged in order of soluble calcium content. The correlation between the returns for lime and the amount of soluble calcium in the soil is very apparent. When it is remembered that these samples have been taken over a wide range of soil conditions the variation in returns from different soils having similar amounts of soluble Ca is exceptionally small, and the curves would seem to indicate a very close relation between the amount of soluble calcium in 0.04  $N$   $H_2CO_3$  and the returns to be expected from liming.

### SUMMARY

The amount of calcium soluble in 0.04  $N$   $H_2CO_3$  has been determined on soils from experiment fields located in seven corn belt states.

The results have been compared with acidity determinations made by the Truog test, the pH value, and also with the returns obtained from the use of lime on these different experiment fields.

The crop increases from the use of lime have been greatest, and have been large enough to be considered practical, on soils where the amount of calcium soluble in 0.04  $N$   $H_2CO_3$ , has been less than approximately 700 lb. an acre in the surface 7 inches or 1300 lb. in the surface 12 inches.

Soils having more than these amounts have usually given medium to small returns for lime, and in most cases have produced all of the common field crops satisfactorily without the use of lime.

So far as this work has progressed the determination of the amount of calcium soluble in 0.04  $N$   $H_2CO_3$  has been a much more reliable method than soil acidity tests, or pH determinations for determining the practical needs of soils for lime.

### LITERATURE CITED

- (1) Duley, F. L. 1924. Easily soluble calcium of the soil in relation to acidity and returns from liming. *Soil Sci.* 17: 213.
- (2) Fleetwood, J. R. 1925. Easily soluble calcium of the soils as an indicator of their response to liming. *Soil Sci.* 19: 441.

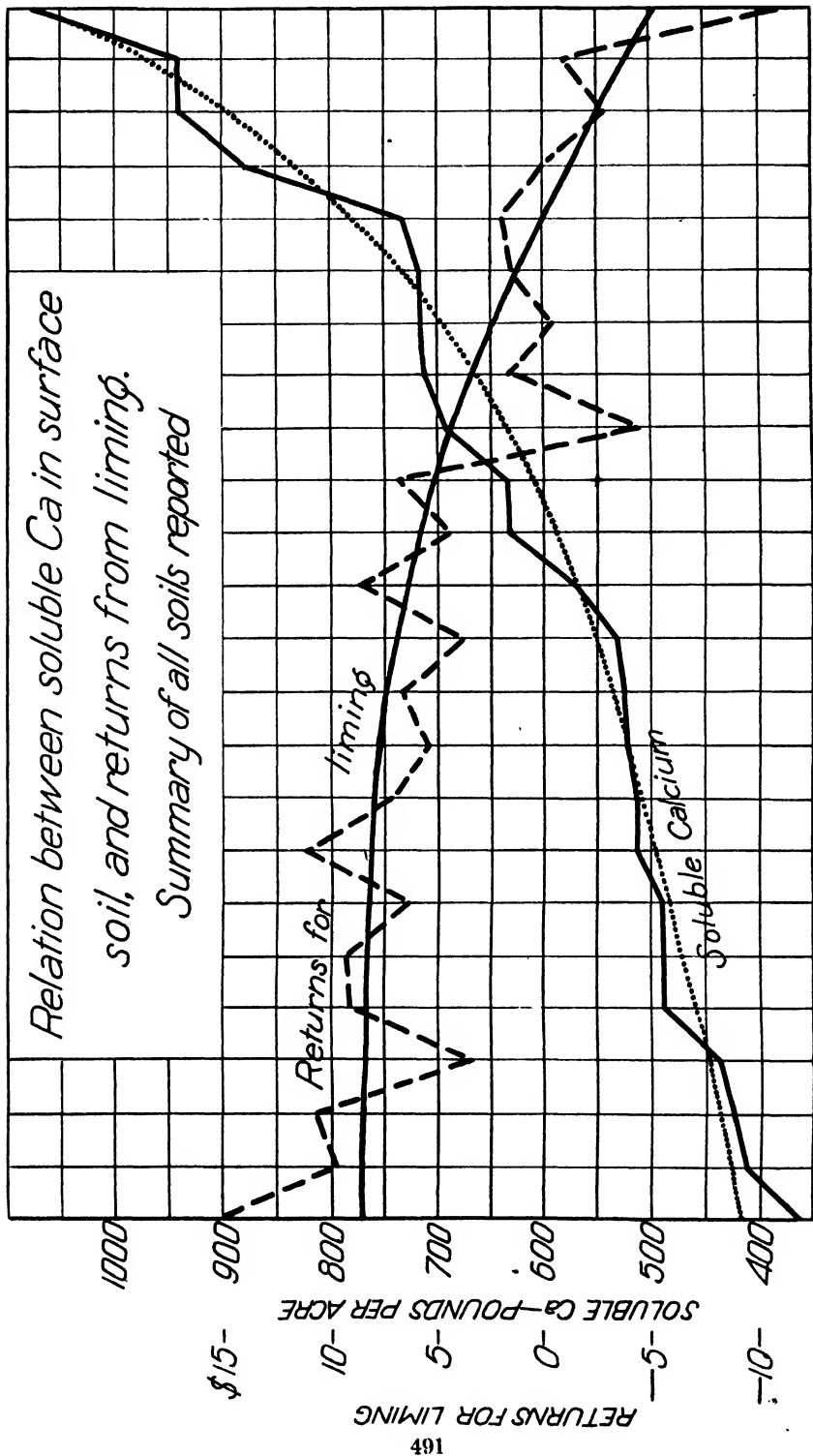


FIGURE 4.—Actual and smooth curves showing relation between calcium soluble in 0.04 N  $H_2CO_3$  and returns from liming on all soils reported

# INVESTIGATIONS IN THE MANAGEMENT OF MICHIGAN MUCK SOILS FOR GENERAL CROP PRODUCTION

P. M. HARMER

*Michigan State College, U. S. A.*

## INTRODUCTION

Different investigators have estimated the extent of muck<sup>1</sup> land in Michigan from 3,000,000 to 4,000,000 acres. Survey of the state has brought out the fact that these muck areas are widely distributed and generally in fairly small areas, so that a considerable proportion of the farms of the state contain some muck within their bounds. Further, investigation has shown that these mucks are not all similar in type but vary in lime content, depth and response to fertilization. While the muck areas of southern Minnesota and southern Wisconsin are uniformly high in lime content, those of southern Michigan, and extending southward to the limits of the Late Wisconsin stage of glaciation into northern Indiana and Ohio, vary considerably in lime content, the low-lime deposits being much in the minority but intermingled among the high-lime areas.

Agricultural utilization of these muck lands has not always met with success. In fact the proportion of failures in muck farming without doubt has been considerably higher than has that on mineral soil. The explanation probably lies in the fact that the attempts at muck farming were made with methods adapted to the farming of mineral soils.

Satisfactory crop production on muck land depends on several factors among which the most important are:

1. The type of muck.
2. The extent of drainage.
3. The cultural methods used.
4. The adaptation of the crop to the soil.
5. The fertilization.

To these should be added the external factors of wind injury (1), disease and insect injury, and the seasonal climate. On mineral soil one or two

<sup>1</sup> In the early days of muck land reclamation the term "muck" was used in Michigan to designate the more decomposed organic soils, while the term "peat" referred to those in a more raw condition. The term "muck" has proven so much more popular, however, that its meaning has gradually been enlarged to include all organic soils, when considered from an agricultural standpoint.

of these factors may be of considerable importance and the remainder of minor consideration. On muck soil, however, all are so intimately related to crop production that the lack of consideration of any one of them may result in complete crop failure.

### THE LIME CONTENT OF MUCK SOIL

In a grouping of the various types of muck, the constituent of the soil which plays the most important part is the lime content. Fortunately the native vegetation is to some extent an indication of the degree of acidity of the soil. Thus a shrub vegetation, which may be comprised of a more or less mixed growth of *Cassandra* (*Chamaedaphne calyculata*), blueberry (*Vaccinium*), Labrador tea (*Ledum Graenlandicum*), cranberry (*Oxycoccus*); often intermingled with Sphagnum moss; or a dwarfed forest growth of tamarack (*Larix laricina*); or tamarack and black spruce (*picea brevifolia*); or a barren muck; is an almost definite indication of a low-lime deposit in Michigan. In southern Michigan a sedge deposit is generally high in lime content but in the central and northern parts of the state it is frequently low-lime. A forest growth of black ash (*Fraxinus nigra*) and elm (*Ulmus Americana*), maple (*Acer*), white cedar (*Arbor vitae*), or large tamarack is quite certain to indicate a high-lime deposit, although the maple and white cedar are sometimes found on very sour muck.

In the determination of whether or not a muck will respond to a lime application, the several tests for acidity, which have been used so successfully on mineral soils, have not proven as reliable when used on muck. If the reaction is not acid, slightly or moderately acid by the Soiltest (2), it is certain that no lime is required and that a lime application may depress crop yields. If the reaction is strongly acid, the possible need for lime will depend both on the muck itself and on the crop which is to be grown. Thus clover, sugar beets, peppermint and celery will require a lime application on muck which is not too sour for good yields of potatoes, carrots and onions. If the reaction of the muck is very strongly acid, it is highly probable that any crop grown thereon will be benefitted by liming.

Occasionally we have encountered mucks which are unproductive, not because of lack of lime but apparently because of an excess of it. The chemical reaction of such soil is alkaline, the alkalinity, so far as investigated, being due to a burning off of the muck surface at some time in the past or to the presence of marl at a slight depth below the surface. These mucks contain such an excess of water-soluble salts as to prove toxic to certain crops. Corn, flax, clover, onions and celery are among the crops which proved most susceptible, while sugar beets and peppermint grew satisfactorily on the fields investigated. Corn turns yellow and becomes stunted at one to two feet in height, flax and clover fail to make satisfactory growth, while the tops of the onions turn yellow and

droop, the crop stops growing and the bulbs fail to develop. Because of the similarity in their effect on crop growth of these fields to the alkali soils of regions of low precipitation, we have come to call them "alkali" mucks. In two instances in which the burning was recent and not deep, the toxicity was entirely corrected by a heavy application of manure. In other cases the toxic condition persisted, even though the soil was well fertilized. More nitrogen appears to be needed in the fertilizer mixture on muck in this condition than is required on soil which is not alkaline.

### DRAINAGE CONTROL OF MUCK SOIL

We decided several years ago that a water-level about three feet below the muck surface was best suited for most general crops. Gradually, however, we have come to see the greater importance of a uniform water-level, that is, one which will remain practically stationary after the recession of the water in the spring. Thus we would prefer a water-level uniformly at two feet or at four feet below the surface rather than one which remains at three feet until the root system of the crop is well established and then rises to two feet or drops to four feet for the remainder of the season. Such a uniform water-level can be secured only when a constant supply of water is available in the ditches and the level of that water is under control. In addition the area should be drained by sufficient lines of tile or lateral ditches, placed deep enough to effectively and rapidly drain the muck without excessively draining it, and to serve as a means of subirrigation in time of drought.

Of the different general crops hay is practically the only one which will give better yields with a water level higher than three feet below the surface. A level of two feet gives better yields of timothy and alsike and sweet clover if there is no serious flooding during the spring months.

### CULTURAL METHODS ADAPTED TO MUCK SOILS

Our cultural investigations serve to emphasize the need of compaction. We have come to regard the 30-inch concrete roller, first recommended by the Bremen Station, as the most important implement on the muck, unless that muck is exceptionally heavy or has inadequate drainage. Comparison of the roller and cultipacker has given better results with the roller. Use of the heavy roller immediately after seeding of grain and potatoes has produced higher yields than rolling before planting. When the heavy roller was not used, better yields were gotten if the land was not plowed in one set of experimental plots but the trials were not continued sufficiently long to warrant a definite conclusion.

In the breaking up of our mucks we find that the tractor drawn plow with 18 to 22 inch bottom is most satisfactory. With a 33-inch rigid rolling coulter attached, the plow carries over roots and stumps and few stops are required.

## CROP VARIETIES ADAPTED TO MUCK SOILS

In our trials with different varieties of grain, corn and potatoes on Michigan mucks, we have found that varieties adapted to the more northern latitudes have given the best results. The relatively shorter season on muck, the danger of summer frosts and the tendency toward lodging of the crops are factors which must be considered. Of the oat varieties, Gopher 674 from the Minnesota Station and Iowar from the Iowa Station have yielded best. Peatland, the new Minnesota barley, and Wisconsin Pedigree have outyielded other barleys, while Rosen rye has proven winter hardy on muck. Wisconsin 25 Yellow Dent and Rustler White Dent have led among the corn varieties. Russet Rural potatoes have outyielded other varieties, but White Rurals have given very good yields and a more marketable potato than does the Russet variety.

## FERTILIZATION REQUIREMENTS OF MUCK SOILS

It is impossible to say just how soon after reclamation a given muck soil will respond to fertilization. Reports have come to our attention of fields which remained productive for 10 years after cropping was begun but we believe these were exceptional cases. Generally a response to fertilization is seen in the third crop produced on the newly reclaimed muck and sometimes in the first or second crop. Much depends on whether or not crops of hay were removed before the land was first broken up. Two different instances have been observed in which crops of wild hay had been removed for long periods of years before the land was reclaimed. On the newly broken muck, the soil had become so depleted that potash was of no benefit when applied alone, while phosphoric acid was of only very slight benefit. When both were applied together, a wonderful increase in yield was secured.

Investigation of the older muck soils as to fertilizer requirements brings out three other groups:—those which require potash only, those which require potash and phosphoric acid and those which require a complete fertilizer. Those which require a complete fertilizer fall into the low-lime group. Those which require potash only will in time develop a phosphate hunger. No mucks similar to the group in northwestern Minnesota, which require only phosphoric acid, have been found in Michigan. In some cases phosphoric acid produces greater benefit than is secured with potash, but generally potash is most needed.

For the past five years we have been making a systematic study of muck fertilization with the idea of determining what amounts of potash, phosphoric acid and nitrogen could be most economically used in the fertilization of the various crops on different types of muck. As an illustration of the method of attack and the results secured, Table 1 shows the yields of potatoes for four years from a four year rotation on

an Eaton County deep muck. The results of similar studies with other crops have already been published (3, 4).

TABLE 1.—Effect of various fertilizer applications on yields of potatoes on sweet muck

Ave. duplicate plots No.	Annual fertilizer application * lb. per acre	Total yield—Bushels per acre				
		1922	1923	1924	1925	Ave. 4 years
1-14	No fertilizer	55.0	101.0	94.2	128.0	94.5
2-15	P 300, K 0	53.8	69.5	114.8	89.2	81.8
3-16	P 300, K 100	133.0	237.3	316.2	200.1	221.6
4-17	P 300, K 200	210.3	295.1	384.2	313.0	300.6
5-18	P 300, K 300	225.2	350.1	447.2	347.8	342.6
6-19	P 200, K 300	215.0	301.4	437.2	346.2	324.9
7-20	P 100, K 300	220.8	294.6	353.6	295.8	291.2
8-21	P 0 , K 300	185.1	262.7	293.2	271.7	253.2
9-22	No fertilizer	56.1	123.4	145.5	187.0	128.0

\* P=Acid phosphate (16 per cent phosphoric acid); K=Muriate of potash (50 per cent potash).

## CLASSIFICATION OF MUCK SOILS

In a classification of muck soils, one other factor in addition to these mentioned above, namely, the depth of the muck, should be considered. Others, such as stage of decomposition and color, might be included, but have the disadvantage that they are not permanent. Thus a muck often shows marked change both in decomposition and color within two or three years after reclamation. On the basis of lime content, fertilizer requirement for general crop production and depth, Michigan muck soils may be grouped as follows:

### I. Low-lime group (Degree of acidity; pH less than 4.25).

1. Lime requirement: Lime needed for crop production; 4 to 8 tons per acre.
2. Fertilizer requirement: Nitrogen, phosphoric acid and potash needed.
3. Depth: Generally deep or medium<sup>1</sup> deposits.

### II. Transitional group (Degree of acidity; pH 4.25 to 5.25).

1. Lime requirement: Varies with muck and with crop grown. May decrease after drainage and settling.
2. Fertilizer requirement: Phosphoric acid and potash needed.
3. Depth: Very shallow to deep.

<sup>1</sup> In this classification the terms very shallow, shallow, medium and deep muck are applied to deposits having depths of 1 to 12, 13 to 24, 25 to 36 and 37 or more inches respectively. On newly reclaimed muck these depths should be increased about one-third.

### III. High-lime group.

#### A. (Degree of acidity; pH 5.25 to 7+).

##### 1. Fertilizer requirement:

- (a) Newly reclaimed muck: Generally no immediate fertilizer requirement.
  - x. Very shallow deposits: Muck disappears within a few years.
  - y. Deep, medium and shallow deposits: After one or more years this group falls under (b) or (c).
- (b) Old muck: Potash needed. After several years this muck will develop a phosphoric acid requirement.
  - x. Deep and medium deposits.
- (c) Old muck: Potash and phosphoric acid needed.
  - x. Very shallow to deep deposits.

#### B. (Degree of alkalinity; pH 7+).

- 1. Fertilizer requirement: Application of manure and growing of green manure as soon as possible appears to be the quickest way to render productive. Complete fertilizer probably needed when crops can be grown.

### LITERATURE CITED

- (1) Prevention of wind injury to crops on muck land. Michigan Agr. Expt. Sta. Cir. Bul. 103.
- (2) Testing soils for acidity. Ibid. Quart. Bul. 6: 93.
- (3) The muck soils of Michigan—Their management for the production of general crops. Ibid. Spec. Bul. 136.
- (4) The management of Michigan muck soils for the production of onions. Ibid. 168.



# THE RELATION OF SOIL MOISTURE TO CULTIVATION AND PLANT GROWTH

F. J. VEIHMAYER AND A. H. HENDRICKSON

*University of California, U. S. A.*

Studies concerning the loss of moisture from soils and the utilization of water by plants in containers and also in field plots have now been in progress at different places in California for several years. Inasmuch as the summer months when most of the work was done were rainless and further characterized by comparatively high temperatures and low relative humidities, the experiments were carried on under most favorable conditions for such studies. Our results which were obtained in the absence of a free water surface do not agree with some of the older conclusions concerning the movement of moisture in soil. Furthermore, they have an important theoretical bearing on studies concerning the water relations of plants as well as on cultural practices with many crops.

Part of these experiments were conducted in large cylindrical galvanized iron containers, varying in size from 23.5 inches in diameter and 48 inches deep to 27 inches in diameter and 72 inches deep. These tanks contained from approximately 800 to 1800 lb. of soil. Two kinds of soil were used. One of these was classified as a Yolo loam from Davis, and the other as a Yolo clay loam from Mountain View, California.

A series of these containers were used during the summer of 1921 to compare the losses of moisture by evaporation from the surface of cultivated and uncultivated Yolo clay loam soil. The arrangement of the tanks was similar to that shown in Fig. 1. The tanks were placed in trenches so that the surface of the soil in each tank was approximately level with the neighboring soil surface. The tanks were further protected from the sun's rays by suitable coverings fitted over the exposed portion of the trench. Weighings were made by means of suspension scales mounted on a portable derrick.

At the beginning of the experiment all of the soil in the tanks except the bottom 6 inches was raised to its maximum field capacity. As soon as the surface soil was dry enough to be properly cultivated four of the tanks were cultivated at weekly intervals to a depth of 6 inches; four to a depth of 8 inches; and three to a depth of 10 inches. The soil in 11 tanks was left undisturbed except to remove weeds as they appeared. The tanks were irrigated August 17, 1921. The average losses of water during the period between August 17 and November 4, 1921, by evaporation in



FIGURE 1.—Galvanized iron containers used in studying movement of moisture in soils and utilization of water by trees. Portable derrick and suspension scales on right

pounds to the square foot of soil surface from the cultivated and uncultivated tanks are given in Table 1.

**TABLE 1.**—Average accumulated losses of moisture in pounds to the square foot by evaporation from the soil surface. Soil was irrigated August 17, 1921

Treatment of soil	August		September		October	November
	25	29	10	30	8	4
Undisturbed except to pull weeds	4.6 ±0.15	5.4 ±0.15	6.8 ±0.15	8.4 ±0.16	8.4 ±0.13	9.4 ±0.17
Six inch cultivation	4.5 ±0.13	5.4 ±0.2	7.0 ±0.22	8.4 ±0.19	8.4 ±0.24	9.8 ±0.29
Eight inch cultivation	4.8 ±0.23	5.4 ±0.25	6.6 ±0.16	7.8 ±0.19	8.0 ±0.24	9.5 ±0.25
Ten inch cultivation	4.3 ±0.11	5.0 ±0.03	6.0 ±0.05	7.3 ±0.09	7.3 ±0.03	8.7 ±0.03

Apparently there was no significant difference in the amounts of water lost from the cultivated and from the uncultivated soils. The average loss of water evaporation from the uncultivated soil in 80 days was  $9.4 \pm 0.17$ , and the loss from the cultivated soils was  $9.4 \pm 0.16$ . Furthermore, it will be noted that approximately 50 per cent of the total loss which occurred in 80 days took place within the first week after the water was applied. Inasmuch, as these losses took place before the soil could be properly cultivated, the supposed efficacy of a soil mulch in preventing losses, is questionable. These results were substantiated in many other trials with other soils both in containers and in field plots.

The distribution of moisture in the soil in the tanks 80 days after the water was applied is shown in Table 2. The amounts of moisture in this table, as well as those which follow, are given as percentages of the dry weight of soil.

There was little difference in distribution of moisture in the soil in the cultivated and uncultivated tanks. The majority of the loss was largely confined to the upper 6 inches of soil and it should be noted that there was a fairly uniform distribution of moisture below 8 inches even 80 days after irrigation.

The fact that there were relatively large amounts of water in these soils even after long exposure to evaporation was demonstrated in a striking manner. Seeds of vetch and barley were planted in some of the tanks on November 4, 1921. Since the upper 4 inches of soil were too dry to germinate the seed a small amount of water was added to each tank after

**TABLE 2.**—Average percentage of moisture of soil. Tanks irrigated August 17, 1921.  
Soil samples taken November 4, 1921

Treatment	Depth of soil samples in inches					
	0-4	4-8	8-12	12-16	16-20	24-36
Undisturbed except to pull weeds	9.4 ±0.25	15.3 ±0.30	17.7 ±0.18	19.2 ±0.24	19.0 ±0.32	18.7 ±0.41
Six inch cultivation	9.7 ±0.40	18.9 ±0.83	18.9 ±0.37	19.0 ±0.82	20.6 ±0.28	20.0 ±1.59
Eight inch cultivation	8.9 ±0.72	15.2 ±0.72	18.2 ±0.28	17.2 ±1.34	19.8 ±0.20	17.1 ±0.61
Ten inch cultivation	7.5 ±0.52	14.8 ±0.50	16.9 ±0.72	18.1 ±0.78	17.4 ±1.06	18.1 ±0.57

planting. In every case, however, this amount of water was not great enough to wet more than the upper 4 inches of soil. The tanks were covered during rains by means of a canvas drawn over a frame. The crop produced was in every way comparable to those produced in nearby orchards where vetch and barley were used as a winter cover crop. The loss of moisture by evaporation during the previous 80 days had not been sufficient to prohibit the growth of cover crops. Both the vetch and barley made comparable growth in all of the tanks and no differences could be noted between the growth made by the plants in the tanks which had been cultivated and those in which the surface soil had not been disturbed.

Several of the tanks were kept under observation for a period of over 4 years. These tanks were covered during rains to prevent the addition of water to the soil after the initial irrigation. The record from one of these tanks given below which contained Yolo clay loam will serve to illustrate further the fact that although rapid loss follows irrigation the total loss of moisture by evaporation directly from the surface of the soil is relatively slight in amount. A comparison of evaporation and transpiration losses is given in a subsequent paragraph.

The total accumulated loss of moisture at the end of different periods of time is as follows: Loss at end of first week, 12 lb.; 6 months, 29 lb.; 12 months, 35 lb.; 18 months, 40 lb.; 24 months, 44 lb.; 36 months, 51 lb.; and 51 months, 57 lb. The total loss of 57 lb., which is 18.9 lb. to the square foot of surface exposed to evaporation, is equivalent to a loss of about 3.4 inches in depth of water within a period of over 4 years, and sampling the soil in the tank at the end of 51 months showed that all of the soil in the tank was still not reduced below the wilting coefficient.

A similar experiment was carried on during the summer and winter of 1922. Several tanks were irrigated on May 13, 1922 so that the soil was wetted throughout its entire depth and the surface soil was undisturbed until October 26, 1922 except to remove weeds as they appeared. On this date the soil to a depth of 12 inches was removed, placed on a piece of canvas, mixed and replaced in the tanks. Vetch plants which had been grown in gardener's flats were planted in the tanks. No water was applied to the soil and the tanks were protected from the rains. The vetch plants matured, making a growth comparable to that of similar plants which were growing as a cover crop in an adjacent orchard, even though the uncultivated soils had been exposed to evaporation from May 13, to October 26, 1922, a period of 167 days.

In 22 tanks similar to those previously described, studies were made on the losses by transpiration by three and four year old prune (*Prunus domestica*) trees. The results showed that losses by evaporation directly from the soil surface were relatively slight when compared to the losses by transpiration. This fact is shown by the record of one of the four year old prune trees which used 1250 lb. of water from March 1 to November 4, 1922. A similar tank containing the same kind of soil as that on which the prune tree was growing, but left uncropped and uncultivated, lost 28 lb. with in the same period of time. Another experiment with a similar pair of tanks illustrated this point still more decisively. The uncultivated tank lost 35 lb. by evaporation during one summer, while the second tank on which Morning Glory, a common orchard weed, was growing, lost 704 lb. during the same period. A study of our complete records shows that the tank with the Morning Glory lost more than twice as much water in 23 days during the growing season than a comparable but uncropped tank lost in 4 years.

Losses by evaporation from cultivated and uncultivated soil were also studied in field plots. These experiments were carried out at four widely separated localities in California. The plots were located at Davis in central California on Yolo loam; at Mountain View, in the Santa Clara Valley, on Yolo clay loam; at Delhi, in the San Joaquin Valley on Oakley fine sand; and at Whittier in southern California on Yolo clay. The plots were laid out in duplicate and of sufficient size to be cultivated by ordinary farm machinery. Weeds were removed from the uncultivated plots by carefully scraping the surface with a hoe. The cultivated plots were stirred to a depth of 6 inches at weekly intervals. The plots were separated from each other by a strip of dry soil 10 feet wide. Soil samples taken around the perimeter of the plots showed that there was no lateral movement of moisture below the surface. At the end of the experiment no significant differences were found in the soil moisture content between the uncultivated and cultivated plots. It was found that, as in the tank experiments, the losses of moisture were confined to the upper foot of soil.

A typical example is given in Table 3. It is clear that the moisture content below the first foot remained practically unchanged.

TABLE 3.—Average percentages of soil moisture in cultivated and uncultivated plots at Davis. Irrigated August 2, 1921

Depth of soil sampled in feet		Aug. 5	Aug. 8	Aug. 15	Aug. 26	Sept. 23
0-1	Cultivated	22.2 ±0.37	20.2 ±0.65	16.3 ±0.63	15.3 ±0.53	16.2 ±0.52
	Uncultivated	23.4 ±0.28	20.0 ±0.22	17.4 ±0.20	17.9 ±0.28	15.2 ±0.26
1-2	Cultivated	21.1 ±1.01	21.0 ±0.69	20.6 ±0.36	19.8 ±0.32	19.9 ±0.32
	Uncultivated	23.6 ±0.25	21.3 ±0.21	21.3 ±0.37	21.1 ±0.31	20.0 ±0.23
2-3	Cultivated	20.4 ±0.94	19.9 ±0.74	20.6 ±0.54	20.0 ±0.35	21.6 ±0.84
	Uncultivated	21.9 ±0.34	21.9 ±0.25	20.8 ±0.33	21.7 ±0.38	19.5 ±0.45
3-4	Cultivated	16.9 ±0.64	18.5 ±0.86	17.0 ±0.45	17.7 ±0.32	17.1 ±0.26
	Uncultivated	19.4 ±0.74	17.8 ±0.57	17.9 ±0.54	18.1 ±0.36	18.2 ±0.36
4-5	Cultivated	17.2 ±0.97	19.2 ±1.29	17.7 ±0.82	18.6 ±0.59	17.9 ±0.51
	Uncultivated	20.1 ±0.59	19.6 ±0.44	18.2 ±0.69	19.4 ±0.84	19.3 ±0.59
5-6	Cultivated	20.7 ±0.99	20.3 ±1.11	18.0 ±1.31	21.0 ±0.88	20.6 ±0.55
	Uncultivated	21.3 ±0.65	21.9 ±0.58	21.8 ±0.63	22.8 ±1.06	22.4 ±0.83

Results obtained approximately 2 months after irrigation by sampling in 4 inch depths in all of the plots showed that most of the loss was confined to the upper 4 inches of soil. A summary of these results is given in Table 4.

The following experiment will serve to illustrate the slowness of the capillary movement of soil moisture under conditions usually met with in the irrigated sections of western United States. Starting on October 17, 1925, water was run for 12 hours in a furrow in sandy loam soil at Davis which had raised a crop of barley and was consequently dried out to about the wilting coefficient. During the next several days a trench was dug

**TABLE 4.**—Average percentages of soil moisture in cultivated and uncultivated plots in 1921. Samples taken approximately two months after irrigation

Location of plots	Treatment	Depths of soil samples in inches				
		0 to 4	4 to 8	8 to 12	12 to 16	16 to 20
Davis	Cultivated	6.6	15.7	20.1	19.1	18.6
		$\pm 0.37$	$\pm 0.51$	$\pm 0.47$	$\pm 0.28$	$\pm 0.23$
	Uncultivated	8.6	15.5	19.0	19.0	19.4
		$\pm 0.39$	$\pm 0.23$	$\pm 0.41$	$\pm 0.21$	$\pm 0.21$
Mountain View	Cultivated	4.0	9.5	10.2	10.3	10.7
		$\pm 0.20$	$\pm 0.41$	$\pm 0.34$	$\pm 0.37$	$\pm 0.41$
	Uncultivated	3.9	9.1	10.4	11.0	10.9
		$\pm 0.15$	$\pm 0.28$	$\pm 0.36$	$\pm 0.67$	$\pm 0.79$
Delhi	Cultivated	1.3	3.9	4.1	4.1	4.2
		$\pm 0.06$	$\pm 0.12$	$\pm 0.12$	$\pm 0.10$	$\pm 0.15$
	Uncultivated	1.5	3.1	3.3	3.7	4.1
		$\pm 0.05$	$\pm 0.06$	$\pm 0.06$	$\pm 0.09$	$\pm 0.25$
Whittier	Cultivated	4.1	11.5	15.1	16.2	15.9
		$\pm 0.22$	$\pm 0.33$	$\pm 0.54$	$\pm 0.31$	$\pm 0.23$
	Uncultivated	4.1	11.0	16.2	17.3	16.5
		$\pm 0.21$	$\pm 0.28$	$\pm 0.43$	$\pm 0.39$	$\pm 0.47$

at right angles to the direction of the furrow and the line of demarcation between wet and dry soil was carefully noted. A system of coördinates was marked on the face of the trench and measurements were taken from these to the boundary of the wetted area on October 22, 1925. Soil samples were taken in the face of the trench which, just previous to the sampling, was sliced off so that a fresh surface of soil was exposed. The results of this sampling and the outline of the wetted area are shown in Fig. 2. The values given in the figure are the ratios of the moisture content found, to the moisture equivalent of the samples. We believe that under the conditions at Davis where this work was done the moisture equivalent is a fair measure of the maximum field capacity of these loam soils. This is clearly indicated in Fig. 2. The difficulties of accurately determining the moisture equivalent and the moisture content of samples are such that a difference of 10 per cent in the ratios is not significant. It is clear that the soil throughout the wetted area was up to its field capacity on October 22, and that there was a sharp drop in the ratio just outside the line of demarcation between the wet and dry soil.

The trench was covered with canvas to prevent the entrance of rain and to lessen evaporation. On December 16, 1925, after a lapse of 56 days from the first sampling, the face of the trench was sliced off, the wetted

area again mapped and samples were taken in the same manner as those on October 22. The results of these measurements are given in Fig. 3. It will be noted that there has been little change in the ratios of the moisture content to the moisture equivalent in the dry soil surrounding the wetted

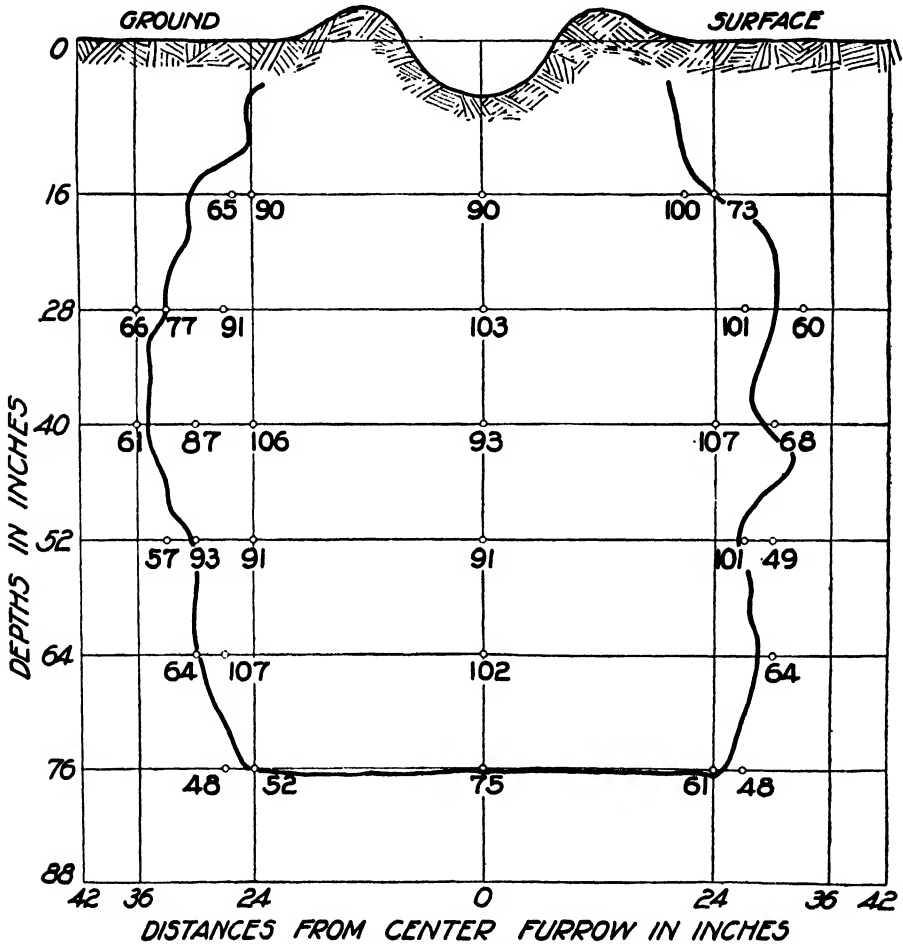


FIGURE 2.—Wet area and soil-moisture contents on October 22, 1925, resulting from the distribution of moisture from a furrow in a loam soil. Water was applied October 17, 1925. The numbers are ratios of moisture contents to moisture equivalents

area and that the ratios within the wetted area are approximately the same as they were on October 22.

A close inspection of the two figures will show that the wetted area has not measurably increased and if Fig. 3 is superimposed on Fig. 2, it will be seen that the line between the wet and dry soil would practically coincide. Of course, the line between the wet and dry soil did fade out to a certain extent since there was a slight movement from around the periphery of the



wetted area but this movement within a period of 56 days was negligible. These results as well as those given concerning the losses of water by evaporation clearly indicate that after the water is distributed in the soil (in the present experiments this usually took place within a period of 48 hr.) further downward movement is negligible. Further adjustment of the

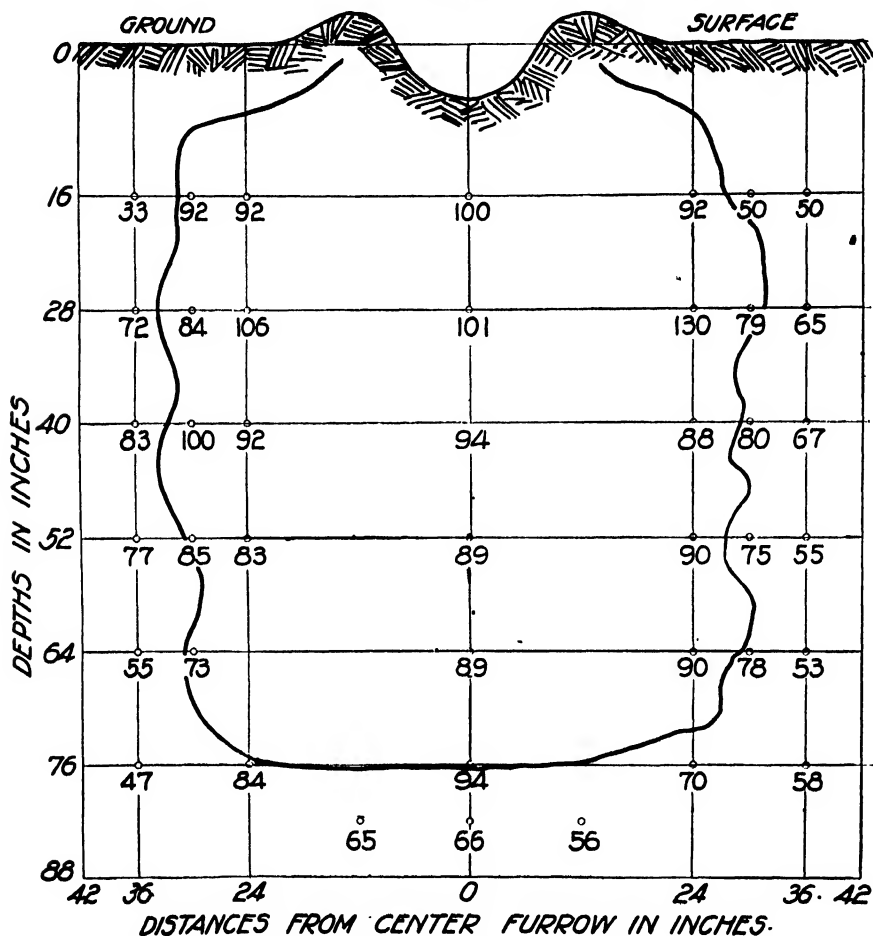


FIGURE 3.—Wet area and soil-moisture contents on December 16, 1925, in the same soil and under the same furrow shown in Figure 2

moisture content between the wet and dry soils which might take place must be at an extremely slow rate.

The old idea that water applied at any point in the soil would be quickly and uniformly distributed throughout the soil has been the cause, in the opinion of the writers, of serious objection being raised to interpretations of much of the earlier work. This is especially true of much of the work done with plants in containers which were supposedly grown under controlled conditions of soil moisture.

In our experiments with trees in containers sufficient water was applied each time the tree was irrigated, to raise the entire soil mass to its maximum field capacity. In this way the possibility of having a moist layer of soil on top with a dry layer beneath was avoided. The method heretofore has usually been to attempt to bring about and, in some cases to maintain, a soil-moisture condition less than the maximum field capacity of the soil. Such experiments were usually made on the basis of maintaining some predetermined soil-moisture content according to an arbitrary schedule which was rigidly observed.

The trees in our experiments were allowed to deplete the average soil-moisture content to different extents before water was again applied. In some cases the moisture content was allowed to fluctuate through a slight range while others were allowed to fluctuate through a much greater range. The upper limit of fluctuation in each case was the maximum field capacity which closely approximates the moisture equivalent in the soils used. The moisture equivalent of the clay loam soil used was about 22 per cent. The use of water by these young French prune trees is shown in Table 5.

TABLE 5.—Amount of water used by young French prune trees as related to leaf area and length of growth

No. of tree	Length growth	No. leaves	Leaf area	Water used Mar. 1 to Sept. 25	Ratio of water use to leaf area	Ratio of length growth to water used
	in.		sq. in.	lb.		
4	536.0	756	3950	782	0.198	0.685
13	363.25	474	2476	544	0.220	0.666
12	214.0	239	1244	316	0.254	0.678
14	221.0	253	1317	328	0.249	0.673
15	333.0	316	1644	427	0.259	0.778
17	372.25	451	2347	587	0.250	0.634
16	365.0	510	2653	572	0.215	0.638
19	491.0	627	3260	712	0.218	0.690

The moisture content for trees 4 and 13 was allowed to fall to approximately the wilting coefficient before adding water; Trees 12 and 14 were kept growing in water logged soil (free water maintained 2.5 ft. below surface) soil; Trees 15 and 17 were grown on soil with moisture content above 16 per cent until the middle of August after which they were allowed to exhaust the soil to the wilting coefficient before more water was added; Trees 16 and 19 were grown on soil kept above 16 per cent moisture throughout the season. The above conditions were maintained throughout the growing season in 1922. It is evident from the foregoing table

that there is apparently no relation between soil-moisture content and either the transpiration per unit leaf area or the length growth.

The rate of use of water, as well as the total seasonal use by the trees, when the soil-moisture content was definitely known, was also studied. Apparently the rate of use of water by the trees was not affected until the soil moisture was reduced to approximately the wilting coefficient. This fact is clearly shown by records from a tree in a tank so arranged that a continuous record could be obtained and also by records obtained by making hourly and daily weighings of other trees. A part of the daily record of one of these trees is given in Table 6 showing the daily rate of loss from the time the tree was irrigated until the soil moisture reached the wilting coefficient. The calculated wilting coefficient of the above soil was about 11.9 per cent.

TABLE 6.—Daily use of water by prune tree in container

1922	Weight of tank	Moisture in soil calculated from weight of tank	Total loss of water through transpiration in 24 hours
	lb.	per cent	lb.
May 19	1120	21.7	
Do 20	1115	21.2	5
Do 21	1107	20.6	8
Do 22	1100	19.7	7
Do 23	1094	18.7	6
Do 24	1088	18.0	6
Do 25	1081	17.1	7
Do 26	1073	16.2	8
Do 27	1066	15.4	7
Do 28	1061	14.8	5
Do 29	1055	14.1	6
Do 30	1050	13.5	5

Throughout these experiments it was found that the soil moisture content corresponding to the calculated wilting coefficient of the loam soils used was a critical condition in the process of use of water by plants. The trees in the containers invariably wilted when this condition was reached, and did not recover until water was added to the soil. This result was likewise observed many times in experimental orchard plots and in commercial plantings.

The behavior of stomata on peach, apricot and prune trees was studied and was likewise found to be decisively influenced by the amount of moisture in the soil when the wilting coefficient was reached (fig. 4). Above the wilting coefficient, however, no differences in the width of the stomatal openings could be detected between trees on a relatively dry soil and those

on a nearly saturated soil (fig. 5). As soon as the soil moisture reached the wilting coefficient, the stomata did not open so wide and began to close earlier in the day than did the stomata on trees on moist soil. The sto-

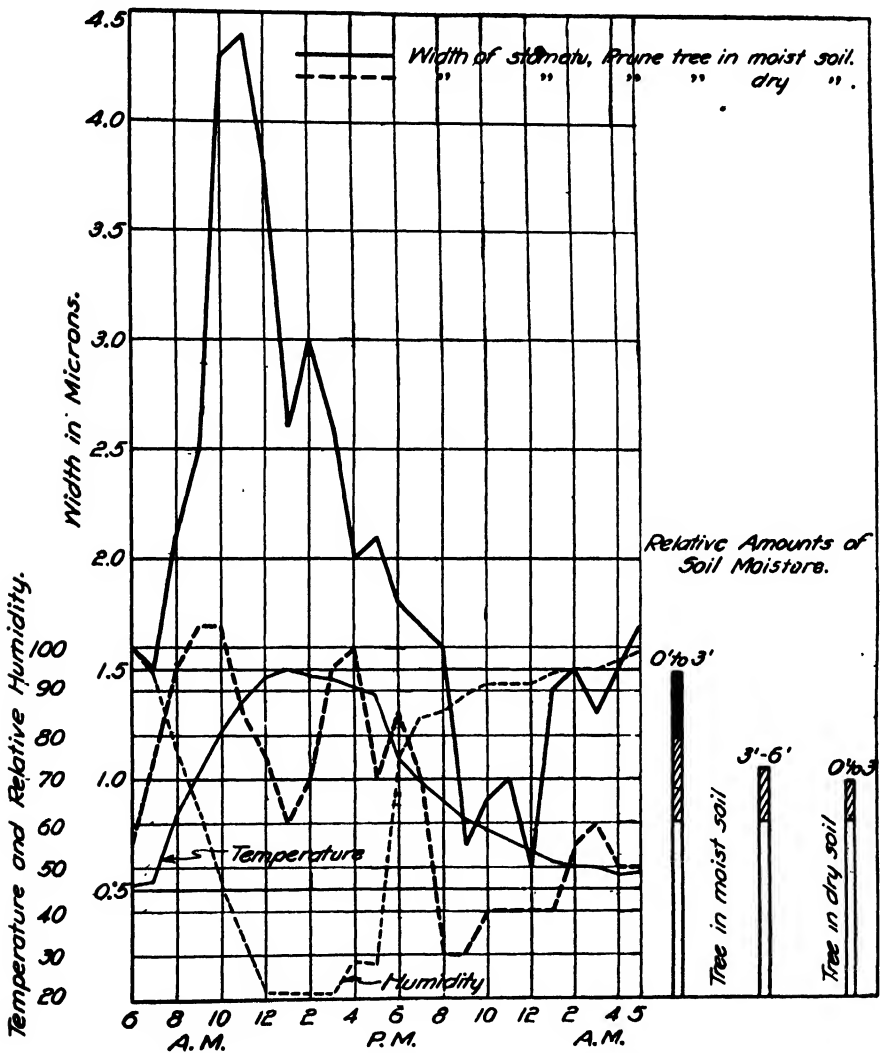


FIGURE 4.—Width of stomatal opening on prune trees, in moist soil and in dry soil at Mountain View, California, September 11, 1924. The relative amount of soil moisture above the wilting coefficient is shown by the solid black column and the relative amount of soil moisture below the hydroscopic coefficient is shown by the unshaded portion

mata on trees on moist soil reached their maximum degree of opening between 9 A.M. and 12 M., while those on trees on soil at or near the wilting coefficient reached their maximum several hours earlier. Ordinarily the greatest closure occurred between 9 P.M. and 11 P.M. Records of

transpiration made on the same trees, but several years previous, indicated that the curves of transpiration and width of stomatal opening are approximately parallel but that transpiration decreased before the stomata began to close.

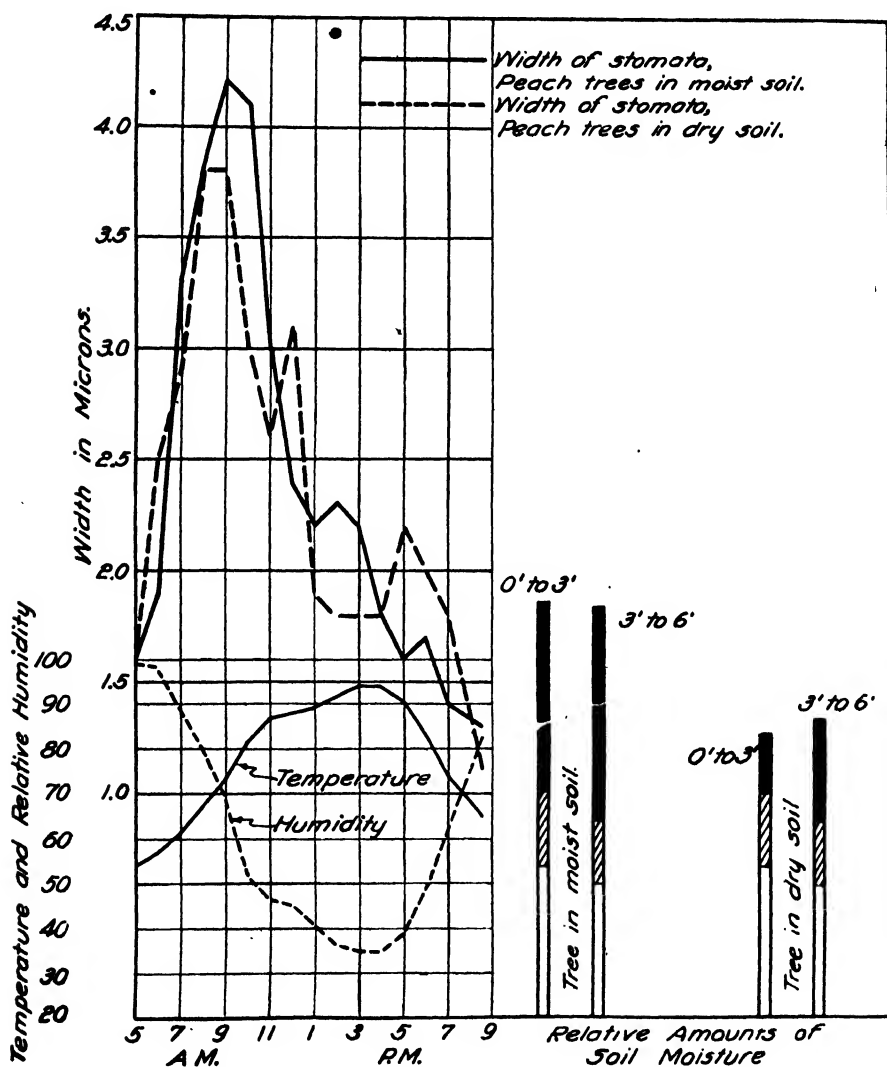


FIGURE 5.—Width of stomatal opening on peach trees in moist soil and in dry soil at Davis, California, July 9, 1925. The relative amount of soil moisture above the wilting coefficient is shown by the solid black column and the relative amount of soil moisture below the hygroscopic coefficient is shown by the unshaded portion

The behavior of the stomata in addition to being affected by soil moisture when the latter reached approximately the calculated wilting coefficient, was also influenced by the position of the leaf on the tree and by

the amount of light reaching the surface of the leaf. Stomata on terminal leaves on long shoots did not open so wide as those on spurs near the base

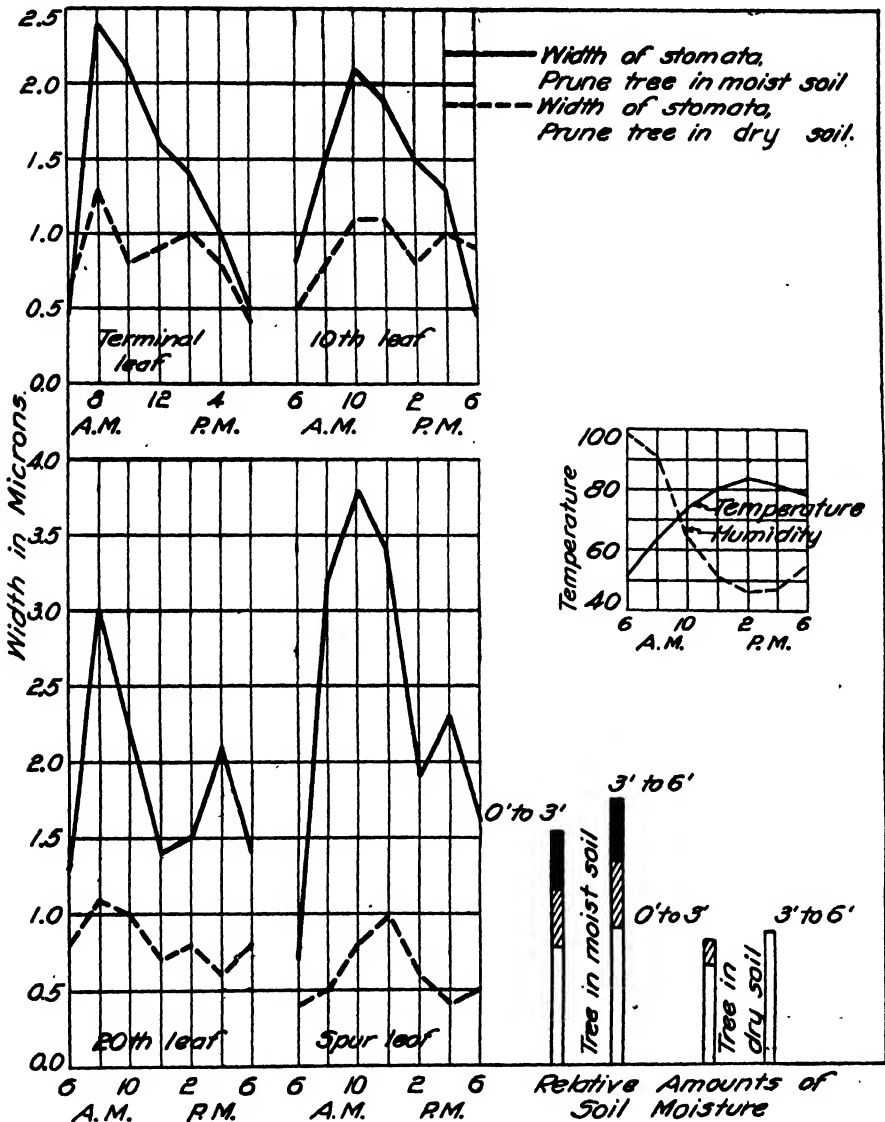


FIGURE 6.—Width of stomatal opening on leaves on different parts of strong shoots and on spurs on prune trees in moist soil and in dry soil at Davis, California, September 15, 1925. The relative amount of soil moisture above the wilting coefficient is shown by the solid black column, and the relative amount of soil moisture below the hygroscopic coefficient is shown by the unshaded portion

of the branch (fig. 6). Stomata on trees shaded by heavy muslin cloth, but well watered, opened sluggishly following sunrise and did not attain

the maximum degree of opening as those on leaves exposed to direct sunlight.

The stomata on the trees studied opened rapidly in response to light during the hours immediately following sunrise. Closure of stomata began before the period of greatest light intensity was reached, probably because of lack of turgidity of the guard and surrounding cells. Turgidity was regained during the night and the progressive opening of the stomata is shown in Figs. 4 to 6.

It was evident from studies with the moisture content of the various parts of the tree during the daylight hours that there was sufficient decrease in the moisture content to account for lack of turgidity in the leaves and the subsequent closure of the stomata. Samples of leaves, terminal and basal portions of twigs, trunk and roots were taken at three hour intervals and dried to constant weight. The bark was separated from the wood and separate determinations made on each. The moisture content of all parts was found to decrease rather sharply in the forenoon and this decrease could be detected in all parts even back as far as the roots by 9 A.M. This loss of moisture was partly replenished between 3 P.M. and 6 P.M.

While the fluctuations in moisture content in the succulent portions of the tree were large throughout the experiment, during the latter part of the summer, it did not go below a certain minimum regardless of favorable external conditions for evaporation. If the moisture content of leaves, bark and wood may be taken as a measure of maturity, the results obtained in this study show that, with the peach trees studied, maturity was reached approximately the same time by trees on moist soils as by those on the drier soils.

No differences in the time of maturity could be detected between trees grown on soils kept continuously moist late in the season and those grown on soils which had practically depleted the available moisture. The trees which were irrigated late in the season shed their leaves as soon as the unirrigated trees. Both the irrigated and the unirrigated trees blossomed at approximately the same time the following spring.

Furthermore, no winter injury was apparent on the irrigated trees, although the winter temperatures were fully as low as usually found in this region.

These results indicate that high moisture content in the soil late in the growing season does not necessarily mean that maturity of new growth and fruit buds on peach trees is delayed. It also casts doubt on the belief that high moisture content late in the season is the principal cause of injury during the winter because of immaturity of the wood. It is probable that other deciduous fruits will be found to respond in the same manner.

The results of these investigations have been put to practical use by many fruit growers in California. Cultivation of orchards is carried on

for the destruction of weeds; for the incorporation of vegetable matter in the soil; for the preparation of a sufficient mulch to facilitate the application of irrigation water at a later date; to facilitate the harvesting of certain crops which are allowed to fall to the ground but not with the idea of influencing the capillary movement of moisture. Furthermore, many commonly accepted ideas regarding the response of fruit trees to different soil moisture conditions are being seriously questioned. Studies, as yet unpublished, seem to indicate that peach trees may be subjected to widely varying ranges of soil moisture, and may even be subjected to severe drought conditions for considerable periods without as marked effect on the fruit as is ordinarily thought to be the case.



# EFFECT OF BURNING THE FOREST FLOOR UPON THE PRODUCTIVITY OF JACK PINE LAND<sup>1</sup>

F. J. ALWAY

*University of Minnesota, U. S. A.*

## INTRODUCTION

When forest land is cleared for agriculture the brush and smaller limbs, along with any dead or down timber of no immediate value, are thrown into piles and burned. The stumps are then removed either by blasting, or by means of stump pulling machines, the land broken and put into crop, or in the delayed system of clearing, which is more commonly practiced, clover is seeded among the stumps and the field used for some years as a pasture, at the end of which time many of the stumps may be pulled directly by a man and team without the use of machine or explosive. The proportion of stumps that thus decay and become easily removable depends upon the character of the original forest. In the course of burning the brush piles more or less of the forest floor is unavoidably destroyed but the extent of this can largely be controlled, and it would be much reduced if it were considered that the burning seriously lowers the future crop producing power of the soil. As to whether it does have such an effect there are no satisfactory data, the opinions entertained being based upon incidental observations and not upon the results of experiments. A few well formulated and carefully executed experiments on different soil types with widely different forest types should go far towards answering these questions. Field experiments started on areas that have been swept by a forest fire are likely to be far less satisfactory than those initiated on well chosen sites in unburned forest. This is well illustrated by the case of the Duluth Experimental Farm, which lay in the path of the fierce forest fire of October 12, 1918, and on which all the standing timber, about 70 acres, was burned over, leaving no unburned tract which might be used for comparison.

## EXPERIMENTAL

With the object of determining whether the productivity of the light soils, naturally occupied by Jack pine (*Pinus banksiana*), are lowered by the processes of burning incidental to clearing, an experiment was started in 1926 near Bemidji, only a few hundred yards from the Bemidji Sand

<sup>1</sup> Published with the approval of the Director as Paper No. 683 of the Journal Series of the Minnesota Agr. Ex. Sta.

Experimental Fields of the University of Minnesota, which have been under operation for the past five seasons. The latter lie just to the east of a Jack pine grove of 30 acres and the experiment was laid out in the west portion of this grove adjacent to a field that had been cleared and cultivated for 11 years, during the course of which it had received very little manure. The only trees that had been removed from the grove were the few Norway pine (*Pinus resinosa*) and white pine (*Pinus strobus*) that were originally scattered through it and such of the Jack pines as had died. The most of the trees were 40 to 50 years old.

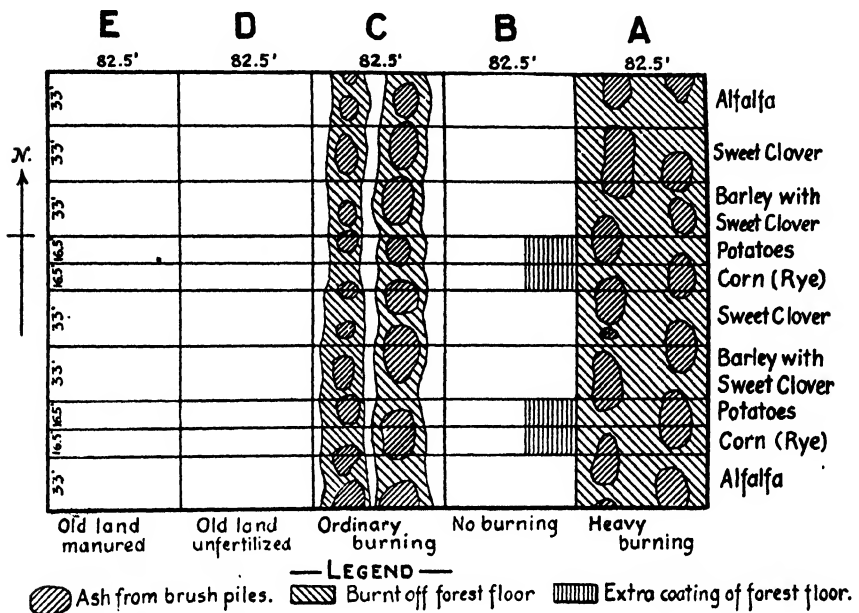


FIGURE 1.—Diagram of experiment showing arrangement of crops, location of piles of brush burned and extent of burning

A rectangle, 20 rods by 16 rods, was selected where the stand of trees and the forest floor were uniform. This was staked out into three half-acre blocks, each 16 rods by 5 rods (fig. 1). Felling of the trees was not started until early in January, 1926, but five months later the land had been freed of all stumps, plowed and put into crop.

The land devoted to this experiment consisted of five half-acre blocks, A, B, C, D and E, which it was planned to till alike after the seeding in 1926 and each season to crop alike. Block A was to represent the heaviest burning feasible in clearing such land. After the brush piles had been burned and the fire had run over as much of the forest floor outside of these as it would, all remaining leaf litter, vegetable mold and other material of organic nature was to be gathered into piles by garden rakes and burned. The stumps alone on all the blocks were to be spared from

the fire, they being too valuable for domestic fuel. Block *B* was to be protected entirely from fire, the brush from the trees on it being burned on the adjacent blocks. Block *C* was to be subjected to much the same methods as those commonly practiced by the farmers in clearing their Jack pine land. Blocks *D* and *E* were on the adjacent old land that had been farmed for 11 years. *D* was to receive no manure or commercial fertilizer but *E* was to be given, just before plowing, 20 tons per acre of manure from a dairy barn where alfalfa and grain were being fed.

During the winter and the early part of the spring the timber was removed and the brush placed in piles in two north and south rows on Block *A* (fig. 2) and in two similar rows on *C*, with none on *B*, half of the



FIGURE 2.—Block *A*, on May 8, 1926, looking north from south edge, and showing piles of brush on either side. Part of the stumps had not yet been blasted out

brush from which was added to the west row of piles on *A* and half to the east row on *C*. In the first part of May, the stumps were blasted out and all removed from the plots without burning. On May 13 the brush on *C* was burned without the fire overrunning all of the block between the two piles or between these and the adjacent blocks (fig. 1). On account of the dry weather prevailing, it was inadvisable to try to burn the piles on *A* at that time, lest the fire should escape into the uncleared woods on three sides. The brush on *A* was burned in small portions during the evenings of May 17 to 26, in the course of which the fire overran the parts between the piles, leaving nothing to be gathered up with rakes to be burned. The entire forest floor was at least as completely consumed as it would have been by a forest fire of the intensity of that of October 12, 1918.

The ash from the piles on *A* was scattered over the rest of the block before plowing and the same was done in the case of *C*. A composite sample of ash collected after burning the brush on *C*, ignited in a

muffle and digested with hot dilute hydrochloric acid for half an hour, showed the following composition: Insoluble residue 77.27 per cent, CaO 8.29 per cent,  $K_2O$  2.97 per cent,  $P_2O_5$  2.19 per cent,  $SO_3$  0.35 per cent.

On Block *B* there was no burning. Just before plowing it an extra coating of forest floor was spread evenly on the east 33 feet of the strips that were later to be planted to potatoes and corn, an area in all of 8 square rods. The material for this had been raked up on an area of 8 square rods in the adjacent woods at a place where the floor appeared the heaviest. This was weighed, sampled and analyzed. The amount of dry matter was found to be equivalent to 11.25 tons per acre of oven-dry material, which contained 54.78 per cent volatile matter, 0.82 per cent N and 0.79 per cent CaO. This would be equivalent to 6.16 tons of volatile matter, 174 lb. of nitrogen and 168 lb. of lime per acre.

During the first week of June all five blocks were plowed. Just before this 20 tons per acre of manure from a dairy stable, where the cows were fed alfalfa and grain, was applied to Block *E*. To avoid further delay the numerous roots thrown up by the plow on *A*, *B* and *C* had to be hauled off the plots, they being too green to burn at once. The land was worked down thoroughly with disk, smoothing harrow and cultipacker. Inoculating soil from a nearby alfalfa field was applied at the rate of 2 tons per acre to all the strips to be sown to alfalfa, sweet clover alone or sweet clover with barley and marl applied at the rate of 2 tons per acre, to only the strips to be sown to alfalfa.

The trial crops selected for the experiment were potatoes, corn, oats, rye, sweet clover and alfalfa. The first represents the extreme of tolerance towards lime-deficiency and the last the extreme in intolerance, while corn is especially sensitive to any lack of nitrogen. All these crops are commonly grown on such sandy lands in that part of the state. The following shows the succession of crops as planned:

1926	Corn	Potatoes	Barley	Sweet clover	Alfalfa
1927	Rye	Oats	Sweet clover	Corn Potatoes	Do
1928	Sweet clover	Corn Potatoes	Rye Oats	Do	
1929	Corn	Potatoes	Rye Oats	Sweet clover	Do

## DESCRIPTION OF THE SOIL

The experiment here dealt with and the older experimental fields are located upon Nymore loamy sand, a soil type that has been developed on the level outwash plain of the glacial Mississippi River. Toward grasses, cereals and root crops this is as lean, i.e. drouthy and low in nitrogen, as any type upon which agriculture is being successfully conducted in the state. With alfalfa (fig. 3) which gradually develops a deep root system, and hence is not so dependent upon the frequency of the rains, it gives relatively much better yields, provided the supply of mineral nutrients is properly cared for (Table 1).

The coarse texture of both soil and subsoil of this type is shown by the data from four representative fields, all virgin, reported in Table 2. For comparison similar data are given for virgin fields on the nearest typical heavier soil type—Nebish loam, which has developed on the till plain of the Late Wisconsin glaciation and is covered with hardwoods.

In nitrogen content the two types show no important difference in the successive levels (Table 3). The surface section, as collected, did not include the forest floor, or leafmold, this having been carefully removed in preparation for the sampling.

On the five blocks of the experiment sampling of the surface soil was carried out in considerable detail in October. For this purpose the two

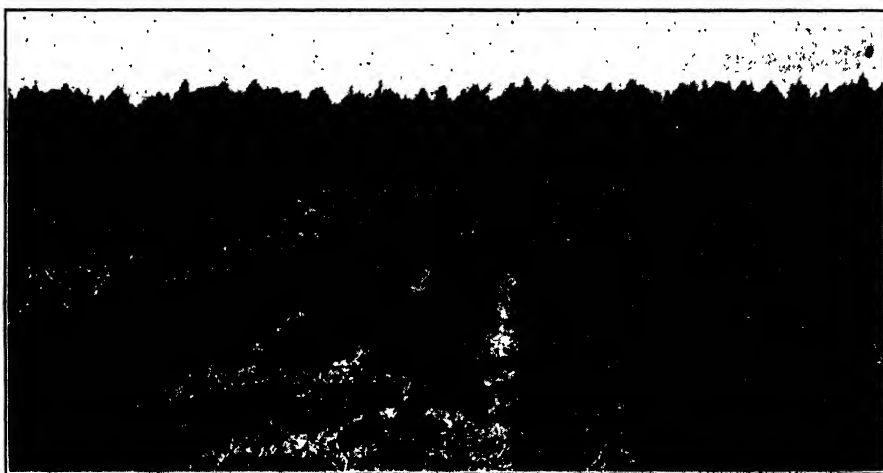


FIGURE 3.—First cutting of alfalfa on Bemidji Experimental Field in 1925, a season with favorable weather. Jack pine grove in background

strips that had been in potatoes were selected, as on these there had been the most cultivation and the soil was the most uniformly mixed. After the potatoes had been dug these strips were spring toothed twice and harrowed twice so as to make the surface soil on each more uniform. Then for each of the 12 samples 50 even cupfuls from as many different places, well distributed over the plot, were taken and mixed. In the case of *B*, samples were collected from the portion where the extra application of forest floor had been made as well as from the part where only the natural forest floor had been plowed under. The nitrogen content was low (Table 4), falling below 0.080 per cent in all but three samples, those from the south part of *B* and *C*. On the unmanured old land, *D*, it was similar to that on the north strip on the blocks just brought under cultivation.

All the moisture equivalents fall between 6 and 8, except on the portion of the south block which received the extra coating of forest floor.

In acidity the newly cleared blocks do not differ distinctly from the old land on *D* and *F*. Judging from the growth of the sweet clover in this

TABLE 1.—Yields of alfalfa and medium red clover hay on Nymore loamy sand at Bemidji

Yield and precipitation	1924	1925	1926
Alfalfa, yield per acre, in tons	2.50	3.24	2.71
Red clover, Do	.79	2.54	1.20
Precipitation—April 1 to August 31, in inches	11.43	17.37	10.78
Do preceding 7 months, Do	4.18	8.53	10.68
Departure from normal—April 1 to August 31, Do	-5.29	+.60	-5.94
Do preceding 7 months, Do	-3.71	+.03	+2.46
Do for 12 months, Do	-9.00	+.63	-3.48

experiment and the results obtained with alfalfa seeded in 1922 on limed and unlimed land on the nearby fields with similar soil, these blocks are

TABLE 2.—Moisture equivalents of Nymore loamy sand and Nebish loam

Depth of section	Experimental field	Nymore loamy sand			Nebish loam			
		Field 1	Field 2	Field 3	Field 1	Field 2	Field 3	Field 4
1 to 6 inches	7	6	7	5	12	13	11	8
7 to 12 Do	5	3	4	4	13	16	9	9
Second foot	4	3	4	4	10	20	13	15
Third Do	3	3	2	3	20	20	18	11
Fourth Do	3	2	2	2				

to be regarded as only slightly, if at all, lime-deficient toward alfalfa and sweet clover.

TABLE 3.—Nitrogen content of Nymore loamy sand and Nebish loam

Depth of section	Experimental field	Nymore loamy sand			Nebish loam			
		Field 1	Field 2	Field 3	Field 1	Field 2	Field 3	Field 4
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
1 to 6 inches	0.060	0.058	0.041	0.036	0.048	0.063	0.082	0.048
7 to 12 Do	.041	.018	.022	.020	.025	.033	.020	.020
Second foot	.032	.011	.012	.006	.026	.037	.019	.028
Third Do	.014	.015	.007	.009	.029	.031	.016	.012
Fourth Do	.014	.009	.005	.005				

TABLE 4.—*Nitrogen content, moisture equivalents and reaction of surface soil of the plots*

Block	Treatment	Nitrogen		Moisture equivalent		Reaction	
		North plot	South plot	North plot	South plot	North plot	South plot
		per cent	per cent			pH	pH
A	All of forest floor burned	0.067	0.068	7	8	5.6	5.2
B West part	Natural forest floor plowed under	.060	.087	6	9	5.6	4.8
B East part	Twice natural forest floor plowed under	.070	.120	8	10	5.2	4.7
C	Forest floor partly burned off before plowing	.067	.080	8	7	5.2	5.3
D	Old land. No manure	.070	.060	6	6	5.3	5.6
E	Old land. 20 tons per acre of manure	.067	.060	6	7	5.5	5.4

### THE CROP YIELDS

The corn and potatoes were planted on June 9 and the barley, sweet clover and alfalfa sown two days later. Good stands of all the crops were secured and all appeared vigorous except on the unmanured old land. On *E* many weeds appeared, which depressed the yields of barley, alfalfa and sweet clover, but on all the blocks the corn and potatoes were kept free of weeds by frequent cultivation, accompanied by hand hoeing. The temperature of the summer was favorable, except for the early killing frost in September, but the rainfall was not (Table 6), there being much less than the normal amount of rain and severe drouths extending from the first of April to June 10, and from the first decade of July to August 19.

The barley was harvested on September 8 and the corn cut two days later, just before the first killing frost, and weighed at once. The alfalfa and sweet clover had made their full growth for the season before their yields were determined, on September 17 and October 9, respectively. The data are reported in Tables 7 and 8.

The yields on the three newly broken plots were practically alike in the case of all five crops. The burning had not proven distinctly beneficial or detrimental. Doubling the amount of forest floor on the east portion of Block *B* did not distinctly affect the yields of either potatoes or corn. The unmanured old land gave much the lowest yields with all the crops and the heavily manured block gave as heavy yields of corn and potatoes as were obtained on the new land, while the lighter yields of barley, alfalfa and sweet clover on this may be attributed largely, if not altogether,

TABLE 6.—Rainfall and temperature at Bemidji in 1926

PRECIPITATION						
Day of month	April	May	June	July	August	September
1	in.	in.	in.	in.	in.	in.
2		trace	0.10			0.20
3		trace		0.05	0.50	0.76
4						
5			0.03			
6	0.04					
7			0.07	0.46		0.23
8		trace		0.98		
9				0.48	0.04	0.03
10			1.18			0.40
11				trace		0.19
12		0.08				
13	trace				0.12	0.22
14			0.03	0.14		
15			0.10			
16		trace	0.39	0.30		
17			0.77		0.33	0.10
18		0.13				
19			0.62	0.23	1.10	
20			0.34		0.09	
21		0.24	0.06		0.39	2.08
22	trace					1.11
23	trace	trace	0.03	0.03		0.04
24			0.13			
25						
26						
27	0.02					
28		0.55				
29				0.38	0.07	
30			trace			0.27
31		0.25				
Total	0.06	1.25	3.85	2.98	2.64	5.63
Departure from normal	-1.41	-1.71	-.20	-1.41	-1.21	-3.04

## TEMPERATURES, °F.

	April 38	May 57	June 59	July 68	August 64	September 51
Mean *						
Departure from normal *	-2	+4	-4	0	0	-5

\* Data from Park Rapids, nearest station with temperature record



to the competition with weeds to which they were exposed and from which the crops on the newly broken land were free.

After removing the corn the land was disked and seeded to winter rye, which made little growth before the coming of an unusually early winter. The growth on all the blocks was similar except on *D*, on which it was poorest.

TABLE 7.—Yields per acre of corn and potatoes in 1926. New land, Blocks A, B and C, broken June 1 to 3, 1926. Crops planted June 10

Block	Treatment	Plot	Corn, green	Potatoes	
				Yield	Marketable
A	All of forest floor burned off before plowing	North	tons 8.8	bu. 114.7	per cent 61
		South	9.0	121.1	70
	Average		8.9	117.9	65
B (West part)	All the natural forest floor plowed under	North	9.8	100.5	56
		South	9.8	137.5	69
	Average		9.8	119.0	62
B (East part)	Twice the natural forest floor plowed under	North	9.8	102.7	58
		South	11.0	145.2	73
	Average		10.4	123.9	65
C	Forest floor only partly burned off before plowing	North	8.9	120.5	61
		South	8.4	136.0	74
	Average		8.6	128.2	67
D	Old land. No manure	North	5.6	65.0	46
		South	5.4	66.6	57
	Average		5.5	65.8	51
E	Old land. 20 tons per acre of manure	North	10.8	141.3	70
		South	9.4	103.5	64
	Average		10.1	122.4	67

## POSSIBLE DEPRESSING EFFECT OF PINE NEEDLES UPON CROP YIELDS

The forest floor was not as heavy in this grove as is found in many of the Jack pine woods and for this reason the question arises as to whether the deleterious effect that is often attributed to the freshly fallen pine needles might not have been evident on the crop yields if there had been selected for the experiment a wood in which the forest floor was exceptionally heavy.

TABLE 8.—Yields per acre of barley, alfalfa and sweet clover in 1926. New land, Blocks A, B and C, broken June 1 to 3, 1926. Crops planted June 11

Block	Treatment	Plot	Barley		Alfalfa hay	Sweet clover hay
			Grain	Straw		
A	All of forest floor burned off before plowing	North	bu. 9.6	tons 0.52	tons 0.31	tons 0.73
		South	11.0	0.49	0.31	0.75
		Average	10.3	0.50	0.31	0.74
	B	All the natural forest floor plowed under	North	8.8	0.48	0.27
South			9.6	0.48	0.24	0.70
Average			9.2	0.48	0.25	0.71
C		Forest floor only partly burned off before plowing	North	12.6	0.47	0.31
	South		7.3	0.64	0.31	0.71
	Average		9.9	0.55	0.31	0.70
	D	Old land. No manure	North	5.3	0.35	0.13
South			5.8	0.45	0.13	0.47
Average			5.5	0.40	0.13	0.47
E		Old land. 20 tons per acre of manure	North	6.2	0.64	0.11
	South		7.7	0.50	0.11	0.54
	Average		6.9	0.57	0.11	0.52

In order to determine whether the needles have any toxic effect a greenhouse experiment was started, using boxes filled with a silt loam subsoil very low in nitrogen with which were mixed needles from both Jack pine and Norway pine. These had been collected by the Lake States Forest Experiment Station to represent the total fall on equal areas for a 12-month period. The analyses are shown in Table 9. Both were used in

TABLE 9.—Composition of leaf litter used in vegetation experiments

Constituent	Jack pine	Norway pine
	per cent	per cent
Volatile matter	97.49	97.99
Ash	2.51	2.01
CaO	0.74	0.35
P <sub>2</sub> O <sub>5</sub>	0.13	0.11
K <sub>2</sub> O	0.12	0.15
SO <sub>2</sub>	0.24	0.23
N	0.70	0.42

their original coarse state as well as in a very finely ground form produced by grinding in a ball mill after drying in a water oven. Thus four materials were used: (1) Jack pine, unground; (2) Jack pine, ground; (3) Norway pine, unground; (4) Norway pine, ground. Each of these was used in duplicate at six different rates, equivalent to 1, 2, 5, 10, 20 and 50 tons per acre, in boxes, 12 x 12 x 8 in. Eight boxes of the subsoil to which no needles had been added served as controls. Soybeans, treated with a pure culture of nodule bacteria were first planted. On the boxes given the very heavy applications of needles the stand was as good as on any and the growth was somewhat the best. After just forming pods the plants were largely killed by parasites and so were not weighed. The growth up to that time made it clear that the needles were exerting no depressing effect upon the soybeans.

After stirring the surface of the soil to incorporate the residues of the beans all the boxes were planted to corn, and all were given two applications of urea, each equivalent to 200 lb. per acre of sodium nitrate. The growth of corn on all was satisfactory, there being no evidence that the needles were harmful or beneficial at any of the rates employed.

It is probable that a Jack pine or Norway pine forest carrying as much as 50 tons per acre of volatile matter in its forest floor will rarely be found and if it were most of this would be in a more or less decomposed condition. As 50 tons per acre of the fresh needles showed no toxic effects it is highly improbable that the needles, in the amounts in which they occur naturally in the forest, could prove toxic to the crops planted immediately after clearing and plowing the land without the burning of any part of the forest floor.

# THE CAUSE OF LOW PRODUCTIVITY IN RECENTLY CLEARED CONIFEROUS TIMBER LANDS <sup>1</sup>

R. E. NEIDIG AND R. S. SNYDER

*Idaho Agricultural Experiment Station, U. S. A.*

## INTRODUCTION

The difficulty of successfully farming the cut-over or recently cleared coniferous timber soils for the first few years in Idaho and the Northwest has come to the attention of farmers and agricultural workers for many years. In the timbered areas of northern Idaho there are numerous acres of this land brought under the plow each year. These lands vary, from virgin timber lands from which timber has just been removed, to cut-over lands which have been cleared for several years. It is therefore a problem of no mean economic importance. While accurate crop yields are not attainable, it is generally understood among the farmers that the first crop will be only fair and the next three or four succeeding crops will be very poor. The general understanding is that five or more years farming are necessary before a good yield of crop may be expected under the present system of farming practice. The reason for this condition is given by the farmers that these soils are "Turpentine Soils."

The Agricultural Experiment Station has been interested in the problem for the past ten years. In 1922 Gibbs and Werkman (5) published a paper on the "Effect of Coniferous Tree Products on Bacteriological Activities in Soil." This article reviews the literature on the bacterial activities in timber soils and summarizes their findings. In general they found that, when various tree products were added to normal soils ammonification and nitrification were inhibited. Their conclusion was as follows:

"The results of the entire work indicate that the low fertility, and apparent toxic condition of the Helmer silt loam, is in a large part due to the timber residue. This material collects continuously throughout the growing period of the forest, and due to its slow rate of decomposition has a direct effect upon the beneficial processes in the soil."

The Agricultural Chemistry Department has been interested in this problem for a number of years and a project was initiated which had for its object the determination of the causes of unproductiveness of recently cleared coniferous timber soils. The first problem was to determine

<sup>1</sup> Paper No. 42, published with the permission of the Director of the Idaho Expt. Sta. from the Dept. of Agricultural Chemistry.

whether these soils were low in available plant foods. This was done by conducting a series of fertilizer experiments with a definite soil secured from a cleared timbered area. The soil is classified as Helmer silt loam (10). The soil passes into a yellowish gray to drab compact silt loam to silty clay loam, which usually extends to a depth of 36 to 50 inches. The surface soil contains a relatively small amount of decomposed organic matter and a relatively large amount of small roots, bits of

TABLE 1.—*Helmer series No. 1, Effect of fertilizers upon recently cleared coniferous timber soil*

Treatment	Crop I	Crop II	
	Total straw	Straw	Grain <sup>a</sup>
	grams	grams	grams
Check—sifted	14.7	5.7	2.17
Check—sifted plus CaCO <sub>3</sub>	15.4	6.2	2.80
100 lb. K as KCl	14.9	4.6	0.65
Do K plus CaCO <sub>3</sub>	15.7	4.7	1.55
Do P <sub>2</sub> O <sub>5</sub> as NaH <sub>2</sub> PO <sub>4</sub>	13.5	4.1	0.67
Do P <sub>2</sub> O <sub>5</sub> plus CaCO <sub>3</sub>	15.8	6.1	1.53
Do K plus 100 lb. P <sub>2</sub> O <sub>5</sub>	15.8	5.9	1.68
Do K Do P <sub>2</sub> O <sub>5</sub> plus CaCO <sub>3</sub>	18.0	5.5	1.65
Do K Do N as NaNO <sub>3</sub>	37.9	17.1	7.4
Do K Do N plus CaCO <sub>3</sub>	40.7	15.3	8.47
Do P <sub>2</sub> O <sub>5</sub> Do N	36.7	16.4	4.47
Do P <sub>2</sub> O <sub>5</sub> Do N plus CaCO <sub>3</sub>	41.0	16.2	8.30
Do K Do P <sub>2</sub> O <sub>5</sub> plus 100 lb. N	37.7	13.2	4.87
Do K Do P <sub>2</sub> O <sub>5</sub> Do N plus CaCO <sub>3</sub>	39.9	16.2	7.55
Do N as NaNO <sub>3</sub> (50 lb. additional in 2nd crop)	35.8	16.3	5.77
Do N as NaNO <sub>3</sub> plus 50 lb. additional in 2nd crop plus CaCO <sub>3</sub>	39.8	14.8	6.8
20 T. well rotted manure (10 T. additional 2nd crop)	20.1	7.0	2.23
20 T. well rotted manure plus CaCO <sub>3</sub> (10 T. additional on 2nd crop)	19.0	6.5	3.80
Check—not sifted (roots, etc.)	15.7	7.8	3.80
Check—not sifted plus CaCO <sub>3</sub>	15.2	8.5	3.47

wood and other material from the forest. The date of cutting off the timber was not known, but it had taken place several years previous as the land was grown up to brush, young conifers and the usual timber vegetation indigenous to this region.

The soil was removed from the top 8 inches and sent into the main experiment station at Moscow. It was well mixed and placed in 4-gallon jars for fertilizer treatment in order to determine whether the low yield after clearing and farming was due to a low amount of available plant food or to some other cause.

## TREATMENTS AND YIELDS

The treatments were made up to try the effect of heavy applications of well rotted manure and also additions of potassium, phosphate and nitrate, singly and in combination both with and without addition of calcium carbonate. The pots were planted to wheat in August, 1922 and harvested on February 26,<sup>1</sup> 1923. No attempt was made to determine the weight of grain and straw separately as the climatic conditions were unfavorable to the best growth under greenhouse conditions. Before replanting the pots a second time additional sodium nitrate was added to each sodium nitrate treatment at the rate of 50 lb. per acre. The pots were replanted to wheat on February 24,<sup>1</sup> 1923 and the second crop harvested on August 21, 1923. Grain and straw weights are recorded in Table 1.

The results of fertilizer treatments show little or no effect of additions of potassium, phosphate or lime, but do show marked increases for the nitrate additions. However, an inspection of the photographs in Fig. 1 shows that even though fertilizer treatments did increase the yield markedly over the natural soil, they did not produce the results that would normally be obtained when similar amounts of sodium nitrate were added to an average soil. During growth it was very noticeable that the wheat plants did not stool. They appeared very spindly even on the soil receiving the nitrate additions. These results indicate that the soil responds in a measure to applications of nitrate fertilizer. The heavy applications of well rotted manure show very little effect over the checks, either in the first or second crops.

From the results using heavy applications of well rotted manure, it appears that this form of nitrogen was not made available fast enough for the wheat plant to develop normally. These results indicate a low nitrifying power of the soil. Additional experiments were planned, which it was hoped would clear up this point. The effect of aerating the soil thoroughly for 2 months before planting and inoculation with pure cultures of organisms were both tried.

Since the supply of this soil had been exhausted, another bulk sample was secured from the same locality as the former sample. This soil is called new soil, meaning that it is a virgin sample of cleared timber soil which has not been cropped. The old soil represents the same soil upon which two previous crops have been grown in the greenhouse. The results are given in Table 2.

A study of the above results shows that the effect of aeration is variable in the checks both with and without lime. Aeration for two months in the rotted manure treatments showed an increased yield from both the limed and the unlimed pots when total dry weight was considered. These pots

<sup>1</sup> Editor's note: These dates are inconsistent, but they follow copy.

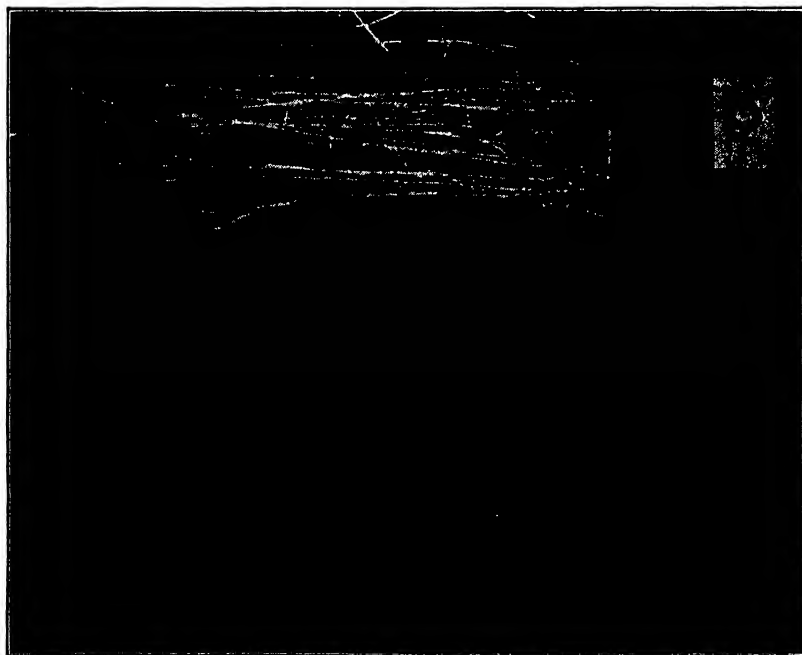
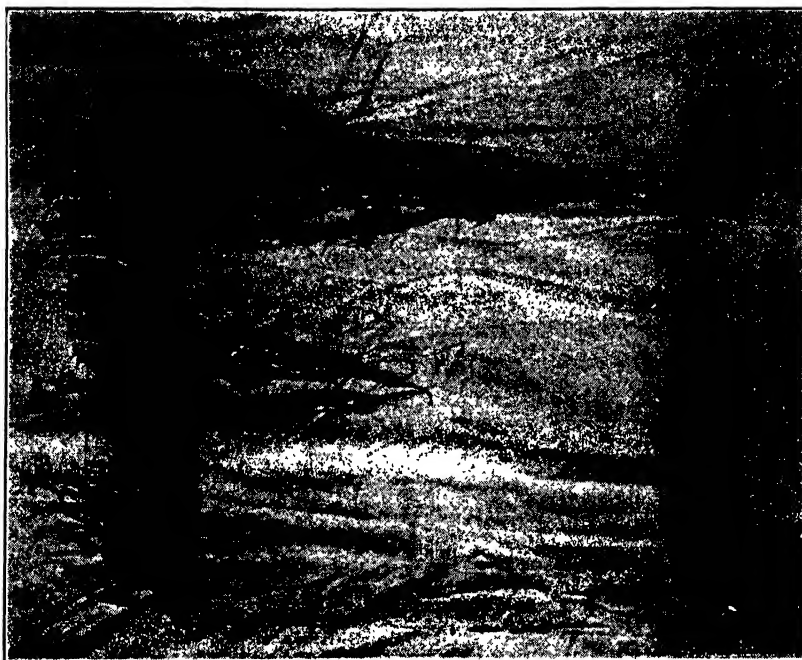


FIGURE 1.—Crop growths and root development from three treatments; (2) Check, sifted; not aerated; (1) Check, sifted, aerated; (70) 20 tons fresh manure and calcium carbonate; inoculated

showed a poor growth of wheat. When we compare the rotted manure treatments with and without the addition of 100 lb. of nitrogen in the form of sodium nitrate we see a great increase in straw with about the same amount of grain. This holds true in both the limed and unlimed treatments.

TABLE 2—*Effect of aeration and inoculation.*

Treatment	No aeration		Aerated 2 months before planting		Inoculated at planting		Inoculated 2 months before planting	
	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain
	grams	grams	grams	grams	grams	grams	grams	grams
Check	11.0	0.17	25.3	3.65				
Check plus CaCO <sub>3</sub>	17.9	5.97	16.9	3.55				
20 tons well rotted manure								
(10 tons additional on 2nd crop)	11.6	0.15	15.7	0.10	19.3	4.35		
20 tons well rotted manure plus CaCO <sub>3</sub>								
(10 tons additional on 2nd crop)	18.4	1.43	27.4	0.80	17.9	5.60		

NEW SOIL								
Check	11.8	4.3			10.6	3.60		
Check plus CaCO <sub>3</sub>	10.7	4.0			10.3	3.26		
20 tons well rotted manure	14.2	5.28			12.0	3.80	57.4	5.10
Do plus CaCO <sub>3</sub>	11.3	3.85			16.7	5.85	47.2	2.35
Do plus 100 lb. N	57.7	4.25						
Do plus 100 lb. N								
plus CaCO <sub>3</sub>	52.6	4.42						
20 tons fresh manure	25.9	7.25	49.6	5.40	27.1	7.40	47.0	4.83
Do plus CaCO <sub>3</sub>	35.4	6.10	49.2	4.00	23.5	8.45	46.3	5.75

## EFFECTS OF WELL ROTTED AND FRESH MANURE

When the well rotted manure treatments are compared with the fresh manure addition, it is seen that fresh manure gives a higher total crop yield than the well rotted manure in the treatments aerated and not aerated. These results amply indicate that the fresh manure contains more plant food (chiefly nitrogen) in an available form than the well rotted manure, and that aeration allows an even greater supply to become available. The fresh manure treatments with 2 months aeration compare very favorably in yield of dry matter to the well rotted manure treatment plus 100 lb. of nitrogen in the form of sodium nitrate.

The effect of both aeration of the soil and inoculation with a pure culture of nitrifying bacteria was next tried to ascertain whether adding nitrifying bacteria would aid in the decomposition of complex nitrogen bodies. New soil was used and the checks for this series are the two treatments inoculated at planting time. A marked effect was noted in the well rotted manure aerated and inoculated 2 months before planting over the treatment inoculated and planted immediately. Whether this result is due to the two months aeration or to the inoculation or both is not known.



These same treatments with fresh manure showed an increase in yield of straw but this increase can definitely be attributed to the greater availability of plant food (chiefly nitrogen) resulting from the 2 months aeration period.

After the crops had been harvested the soil was carefully removed from the pots and the roots examined. The photographs in Fig. 1 show the crop growths and the roots from three treatments. These roots are characteristic of the series. In all cases root development was directly proportional to yield of crop. The check treatments (Virgin Soil) shows clearly an arrested root development. Evidently there is a marked retarding effect on root development in this soil which is partially overcome by heavy applications of fresh manure. The following yield of straw and grain was obtained:

Pot	Straw grams	Grain grams
2	11.0	0.17
1	25.3	3.65
70	46.3	5.75

#### EFFECT OF LIGHT AND HEAVY BURNING OF THE SOIL

The effect of light and heavy burning of the soil was next tried. The soil was lightly burned by burning straw on top of a thin layer of soil in a galvanized can. The heavy burning consisted of maintaining a fire around the can and on top of the soil until it was heated throughout. Sterilization was secured by placing the soil in an autoclave and holding it at 15 lb. pressure for 4 hours. The results are given in Table 3.

TABLE 3.—*The effect of burning and sterilization*

	Straw	Grain
Burned lightly	29.7	2.33
Burned heavily	48.4	4.58
Burned heavily and inoculated	38.0	10.5
Sterilized	50.9	9.61
Sterilized and inoculated	49.5	10.0

The light and heavy burning shows a beneficial result on crop yield. The heavier burning giving the greater yield of dry matter. The dry matter was not increased in the inoculated pots though the yield of wheat was greater. It is not thought that this difference is significant as the difference is no doubt due to difficulty in burning two soils cans similarly.

One naturally would expect burning a soil to increase the available mineral content. This result is accomplished when peat lands are accidentally burned and crop yields are increased the following year (7).

The fact, however, that additions of soluble potassium and phosphorus did not result in increased yields, refutes this theory. Indications from this treatment point rather strongly to the presence of a toxic substance in the soil that is destroyed by burning. Sterilization also increased yields materially. Inoculation and sterilization did not increase the yield over the sterilization treatment, therefore showing no beneficial effect of inoculation. Sterilization would be expected to increase yields due to the breaking down of complex nitrogenous compounds, changing the biological flora in the soil and thereby promoting better soil conditions. Much the same condition takes place in soil that has been burned.

A new series of treatments was commenced on June 27, 1924. Jenkins Club Wheat was planted and harvested November 11, 1924. The treatments are self explanatory and are given in Table 4. They were made on a new bulk sample of soil taken from the same locality as the first bulk sample. Aeration was carried out throughout the growing period by means of a coiled tube inserted at the bottom of the jar with openings along the coil. Air was drawn through for 30 minutes every other day by means of an aspirator.

A study of the results on this series corroborates the results of the first series of fertilizer treatments. It is seen that liming shows little or no effect and that well rotted manure produces very little increase in dry matter over the checks, even when aerated and inoculated. With aeration for two months in the first series considerable benefit was noted on the third consecutive crop. The beneficial result of aeration by aspirating air through the soil during the growing period is not as marked on the first crop grown on virgin soil.

Ammonium sulfate treatments compared very favorably with sodium nitrate treatments. Inoculation or aeration during the growing period did not seem to be beneficial. Complete fertilizer treatments did not produce any greater yields than sodium nitrate alone.

Three pots were treated with gypsum, but these treatments showed no increase in yield over the check pots.

In view of the results obtained on this soil a new series was planned to learn more about the nitrate production and the fate of the nitrate in the soil, whether denitrification takes place in the soil or whether the nitrate production is retarded. In all the previous trials it was observed that well rotted stable manure did not produce the increase in crop that was expected, even after three croppings. It remained to be determined whether nitrification was inhibited because of toxic substance in three products added to soil as shown by Gibbs and Werkman, or whether denitrification takes place in resinous soils as suggested by Koch (6), or whether the presence of cellulose in this soil utilized the nitrate for the fermentation of cellulose as suggested by Anderson (2), and by Viljoen and Fred (9). With these ideas in mind a new series was commenced in



cooperation with the Department of Bacteriology, which had for its object a study of the rate of decomposition of dried blood and ammonium sulfate by the nitrifying bacteria in uncropped soil. Heavy treatments of sodium nitrate were added to determine whether denitrification took place. Inoculation with pure cultures of nitrifying organisms was included in the treatments. Aeration was also tried alone and aeration and inoculation together. Crops were grown on certain of these treatments.

Nitrate determinations were made at frequent intervals and total nitrogen of the soil was determined at the end of the experiment. Cellulose determinations were made on some of the treatments using the methods of Charpentier (4) modified by Barthel and Bengtsson (3). The nitrate determinations were made by the reduction method using Devarda's alloy. Total nitrogen was determined by the A. O. A. C. modified Hibbard method. The treatments were made in triplicate. The treatments and analytical data are given in Table 5.

The yields of crops on the treatments mean very little because the heavy treatments in themselves had a tendency to be toxic to the plant during germination and growth. Nitrate determinations show that nitrates are present in large amounts at the close of the experiment in the treatments receiving heavy sodium nitrate applications. There is a lower amount of nitrate found in the last period of sampling (August 17). Whether this is due to error in sampling or to denitrification is unknown.

The dried blood treatments show a gradual accumulation of nitrates up to the last period of sampling. This is not as rapid as one would expect. Inoculation did not aid in nitrification. The effect of 2 months' aeration is shown in the greater nitrate content at the initial period of sampling. The total amount nitrified was not materially different from the non-aerated pots.

The ammonium sulfate treatments all showed small increased amounts of nitrates at each progressive sampling. Here too is shown a less amount of nitrate on August 17 than on July 17. One possible explanation of this is the fact that the wheat was fully ripened by July 21st, but was not harvested and the soils were not sampled until August 17, the date of harvesting. If denitrification takes place, its greatest effect is in this last period of the experiment. Aeration acts the same in the ammonium sulfate treatment as in the dried blood treatment. It is quite evident that ammonium sulfate changes to nitrate very slowly in this soil.

The determination of cellulose was made on a few treatments and very little cellulose found. This fact eliminates cellulose as the chief factor in poor crop production from this problem.

These results also preclude the possibility of denitrification being the chief cause of poor crops. Our evidence here points to some cause of low crop production other than that of available plant foods, because even

TABLE 5.—Nitrogen fertilizers

Pot. No.	Treatment pounds per acre	p. p. m. Nitrates						Total nitro- gen, Aug. 17	Yield Aug. 17		Cellu.  per cent
		Mch. 17	Apr. 17	May 16	June 16	July 17	Aug. 17		Straw	Grain	
1-3	3600 NaNO <sub>3</sub>		287.3	238.6	285.3	239.8	172.8	0.161			0.0165
4-6	20,000 dried blood		111.3	349.3	348.0	423.0	321.3	.251			.0140
7-9	2800 (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>		27.3	65.0	94.6	147.6	157.8	.160			.020
10-12	3600 NaNO <sub>3</sub>							.159	16.4	8.33	.025
13-15	20,000 dried blood							.258	15.57	5.2	
16-18	2800 (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>							.151	22.47	8.6	.024
19-21	20,000 dried blood (inoculated)		122.0	335.3	356.0	405.9	284.8	.241			
22-24	2800 (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> Do		28.0	75.5	108.0	175.9	146.6	.153			
25-27	20,000 dried blood Do							.243	18.4	7.4	
28-30	2800 (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> Do							.153	18.7	6.87	
31-33	Check		24.0	34.6	24.6	35.4	32.3	.134	13.45	6.72	.0215
34-36	Check										
37-39	20,000 dried blood (aerated 2 months)	388.0	399.3	342.6	296.7	401.6	250.3	.217			
40-42	20,000 dried blood { Inoculated and aerated 2 months	315.3	401.3	393.0	404.6	410.9	302.6	.224			
43-45	20,000 dried blood Do							.224	7.18	2.75	
46-48	2800 (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> (Aerated 2 months)	74.6	130.6	136.0	140.7	161.6	112.0	.151			
49-51	2800 (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> { Inoculated and aerated 2 months	74.6	134.0	176.0	189.5	170.9	115.8	.155			
52-54	2800 (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> Do							.147	14.96	7.07	
55-57	Checks (aerated 2 months)	44.0	42.6	38.5	28.0	28.0	32.7	.134			

when abundant plant foods are supplied, the crop growth does not equal that which would be expected from normal soils.

Since substances have been found in soils which have been shown toxic to higher plants by Koch (6) and others, a study of this soil was begun to ascertain if it contained substances which were toxic to plants when applied in the pure form. Since these soils are known to contain the residues of coniferous trees, the substances expected to be present that might be toxic would be in the nature of resins and oils of various descriptions. Consequently our first search was pointed in this direction.

### METHODS OF EXTRACTION

A large bulk of soil was secured and the roots and fibrous materials were sifted out. Several solvents were tried, but 95 per cent alcohol proved most effective. In the early part of the experiment the Morgan Soil Pressure Extractor was used, but later it was found much easier to use a simpler percolation method. The alcoholic extract of soil was combined, the alcohol distilled off under reduced pressure and the residue taken up with absolute alcohol. After filtering off the undissolved material, the alcoholic filtrate was evaporated to a small volume. This residue was further extracted with ether and then made up to such volume with alcohol that one cubic centimeter of the solution contained an amount of extractive principle equal to that contained in 63 g. of soil.

In a similar manner an extract was made from the roots found in this soil. The purified extract was made up so that one cubic centimeter of alcohol contained the amount of extractive material found in roots from 63 g. of soil. A series of culture solutions were made up as follows:—Coarse sand and gravel were treated with definite amounts of alcoholic extract and the sand and gravel stirred constantly until all the alcohol was evaporated. This procedure was followed in order to secure a uniform thin coating of resinous material on the sand and gravel. The sand and gravel was then placed in a culture flask and nutrient media added. The sand and gravel procedure was used in order to have the maximum surface of the resinous material exposed to the nutrient medium in order that if the extractive material was toxic, its toxicity would show more rapidly.

The control flasks contained sand and gravel that had previously been treated with alcohol and then evaporated, together with the nutrient media. The amount of gravel used in each flask was 630 g. Hence one soil or root equivalent of extractive material was 10 cc. of the alcoholic extract. After standing for a day 4 wheat seedlings were placed in paraffin tops and allowed to grow.

The photograph in Fig. 2 shows the effect of these extracts on the growth of wheat seedlings. Since duplicate treatments were very similar in appearance only one bottle was taken from each series.

The results show that the material from the soil and roots is quite toxic

to wheat seedlings in solution cultures. The amount of material from the roots was much greater than that from the soil, hence there is a greater toxic effect shown.

In order to learn more about the composition of this alcoholic soil and root extract, a portion of the residue was treated and separated into its component parts by the method of Tschirch (8). The alcoholic residue was treated several times with a 1 per cent solution of ammonium carbonate, the ammonium carbonate solution was then filtered and neutral-

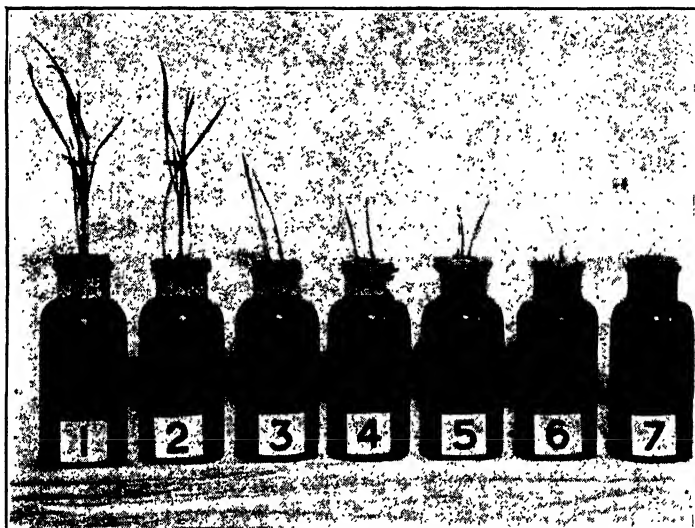


FIGURE 2.—The effect of alcoholic extracts on growth of wheat seedlings

Bottle No.

1. Control
2. Media plus 1 soil equivalent of extractive material
3. Media plus 3 soil equivalents of extractive material
4. Media plus 6 soil equivalents of extractive material
5. Media plus 1 root equivalent of extractive material
6. Media plus 3 root equivalents of extractive material
7. Media plus 6 root equivalents of extractive material

ized with dilute hydrochloric acid and a resinous material obtained. The former residue was next treated in a similar manner with a 1 per cent sodium carbonate solution and a quantity of resinous material obtained after neutralizing with dilute hydrochloric acid. The original residue was then treated a third time with a 0.1 per cent and a 1 per cent potassium hydroxide solution until all material that would dissolve was removed. The combined solutions from the potassium hydroxide extractions were neutralized with dilute hydrochloric acid and the resinous material collected. Owing to the small amount of residue left after these three extractions, no attempt was made to separate the volatile oils from the resinous residue.

The four fractions were repurified by repeating the process above described. Their effect on the growth of wheat seedlings was determined by adding 0.1 g. of each to a bottle containing sand and gravel. The material was first dissolved in alcohol and then added to the sand and gravel stirring constantly until all the alcohol had evaporated. The nutrient medium was then added to the bottles and after standing for 24 hours, 4 wheat seedlings were placed in the bottles, using paraffin holders.

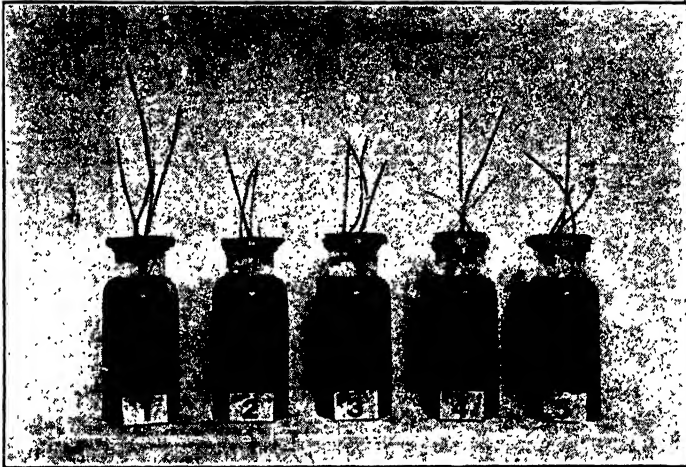


FIGURE 3.—Effect of repurified fractions upon the growth of wheat seedlings

Bottle No.

1. Control
2. Residue remaining after the 3 extractions
3. 0.1 and 1.0 per cent KOH extraction. Used 0.1 g. material
4. 1.0 per cent  $(\text{NH}_4)_2\text{CO}_3$  extraction. Used 0.1 g. material
5. 1.0 per cent  $\text{Na}_2\text{CO}_3$  extraction. Used 0.1 g. material

Another treatment was made using 0.4 gram of each of the purified materials. The nutrient medium was added to the bottle containing the resinous materials and allowed to stand in contact for 7 days prior to planting the wheat seedlings

The experiment was allowed to stand 5 days after which the photographs in Figs. 3 and 4 were taken.

Considerable toxic effect is noted for all resin treatments. One-tenth gram of material showed almost as much toxicity as 0.4 g., indicating that the solubility factor probably enters into the degree of toxicity. The effect of adding calcium carbonate to the solutions and allowing these solutions to stand for 5 days, shaking numerous times daily, was next tried. After standing 5 days new wheat seedlings were placed in the bottles. After 5 days' growth of the wheat seedlings the following photograph was taken. All the residue had been used in the previous experiments, hence it was omitted from this series.



It is seen from the above photograph that lime did not counteract the effect of the resinous materials.

### ULTIMATE ANALYSIS OF THE PURIFIED FRACTIONS

The three fractions of resinous materials were next analyzed by the combustion method. Following are the results:

#### *Analysis of purified fractions*

No.	Carbon per cent	Hydrogen per cent	Oxygen per cent
1. 0.1 and 1.0 per cent KOH extract	76.64	10.17	13.21
2. 1.0 per cent $(\text{NH}_4)_2\text{CO}_3$ extract	71.86	8.76	19.39
3. 1.0 per cent $\text{Na}_2\text{CO}_3$ extract	73.84	9.18	16.83

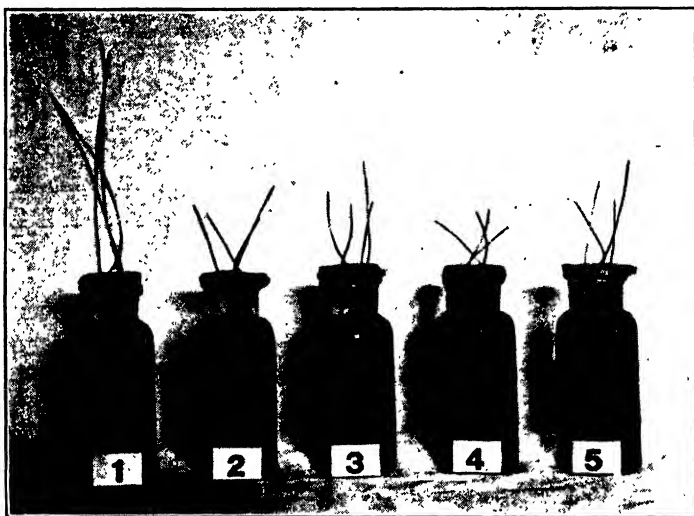


FIGURE 4.—Effect of repurified fractions upon the growth of wheat seedlings

#### Bottle No.

1. Control
2. Residue plus nutrient solution only
3. 0.4 g. Resin soluble in 0.1 and 1.0 per cent KOH
4. 0.4 g. Resin soluble in 1.0 per cent  $(\text{NH}_4)_2\text{CO}_3$
5. 0.4 g. Resin soluble in 1.0 per cent  $\text{Na}_2\text{CO}_3$

Calculating the possible relationships of the carbon, hydrogen, and oxygen we have for Sample No. 1  $(\text{C}_8\text{H}_{13}\text{O})_x$ , for No. 2  $(\text{C}_9\text{H}_{13}\text{O}_2)_x$  for No. 3,  $(\text{C}_6\text{H}_9\text{O})_x$ . The molecular weight determinations were then made by the freezing point method on two of the fractions. From the sodium carbonate extract the value 2130 was obtained and for the ammonium carbonate extract 2603. Calculating a possible for the former would be  $(\text{C}_6\text{H}_9\text{O})_{22}$ , and for the latter  $(\text{C}_9\text{H}_{13}\text{O}_2)_{17}$ . These two formulas are wholly empirical. It is our opinion that these fractions are mixtures of closely

associated resin acids rather than a single compound. Since they have accumulated for ages as residues from all types of coniferous trees it is only reasonable to expect that a mixture of resin acids would be present in the soil.

A paper on "The Effect of Woods and Tree Products on Bacteriological Activities in Soil" (in press), written by Gibbs and Batchelor contains certain data that will be reproduced here. They examined numerous samples of soils collected within a 15 mile radius.

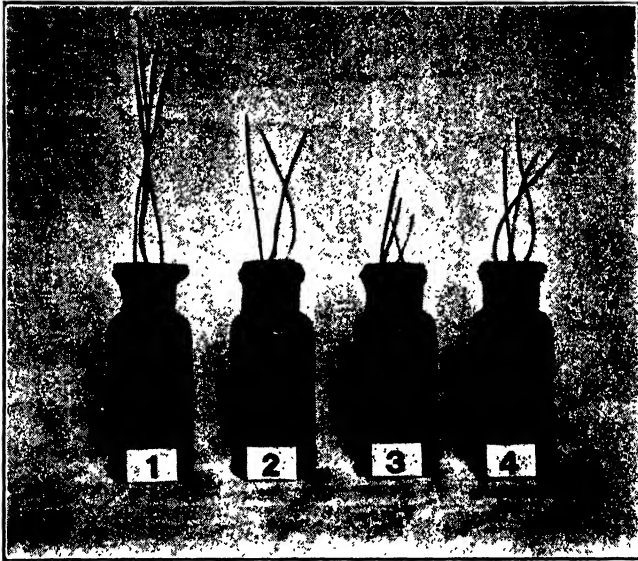


FIG. 5.—Effect of repurified fractions and calcium carbonate upon growth of wheat seedlings.

- |    |  |    |  |
|----|--|----|--|
| 1. | Control plus 5 g. lime   |    |  |
| 2. | 0.4 g. Resinous material soluble in 0.1 and 1.0 per cent KOH plus 5 g. lime. |    |  |
| 3. | 0.4 g.   | Do | 1.0 per cent $(\text{NH}_4)_2\text{CO}_3$ Do |
| 4. | 0.4 g.   | Do | 1.0 per cent $\text{Na}_2\text{CO}_3$ Do     |

A description of three typical soils follows:

Soil No. 26, a virgin soil now growing timber. Soil No. 31 (the soil used by the authors) is from land recently cleared, but still covered with types of undergrowth and second growth conifer timber, and Soil No. 9, a soil which has been under cultivation for more than 5 years. The results on ammonification and nitrification are given in Table 6.

Gibbs and Batchelor conclude—"Thirty-one of the samples were tested for ammonia and nitrate accumulating ability. All showed ability to accumulate ammonia but 16 were decidedly lacking in ability to form nitrate from ammonium sulfate or blood. They were not benefited in this respect by the addition of calcium carbonate. Fifteen of the samples were able to form small amounts of nitrate from ammonium sulfate and

larger amounts from blood in the absence of calcium carbonate. Those soils which were not able to form nitrate were largely soils bearing virgin timber, while those which formed nitrate were largely soils long under cultivation."

TABLE 6.—Ammonification and nitrification of various types of timber land

Soil No.	Ammonification	Nitrification			
	NH <sub>3</sub> per 100 grams soil mg. N	Ammonium sulfate		Blood	
		Unlimed mg. N	Limed mg. N	Unlimed mg. N	Limed mg. N
26	15.3	0	0	0	0
31	22.7	1.4	8.7	3.8	20.2
9	56.7	8.5	29.5	30.5	68.0

These results correlate our findings in that there is a very slow nitrification of well rotted manure in all our experiments by Soil No. 31. It appears that there is an inhibition of nitrification and it is our opinion that these resinous principles are the cause of this retardation of nitrification as well as retarding normal growth of the plant. Various attempts (in cooperation with the Bacteriology department) were made to test out the effect of these purified resinous principles on bacteriological activities, but each trial failed because of the difficulty involved in the addition of resin to the culture solution, since any solution used to dissolve the resin would of itself be harmful to bacteria. It is hoped to complete this phase of the work in the future.

Koch (6) has stated that certain coniferous residues were toxic to higher plants and bacteria. He suggested that "these substances may act as energy for the denitrifying group and thus prevent nitrate accumulation." Pine resin acting in this manner he gave as a reason for the low nitrate content of resinous soils. Nemec (7) has recently demonstrated the low intensity of nitrification in forest soils growing conifers and at the same time the lively intensity of nitrification in soils growing broad-leaved deciduous trees. He has also shown the favorable influence on nitrification of beech underwood on high forests of Scotch pine. Similar results were noted when comparisons were made of close grown forests of conifers with mixed stands composed of broad-leaved species and species with persistent leaves. Many other investigators state that it is not common to find nitrates in any quantity in coniferous forest soils.

The soil used in our study does contain considerable nitrate, around 30 p. p. m., because it has been cleared for a number of years, but the rate of nitrification is very slow. It is believed that these resinous substances are the cause of the retarded nitrification. In the presence of abundant

added sodium nitrate, crop yields are not large, hence we believe both bacterial action and plant growth are effected by the resinous material. Denitrification is not thought to occur to any extent at least, because nitrates added to this soil can be practically entirely accounted for after a few months in the soil.

Neither is it thought that cellulose is the cause of low nitrates in our soil as was found to be true by Viljoen and Fred (9) in studies on sawdust from various woods such as willow, birch, alder, and poplar. Coniferous growths and residues appear to act differently than woods from the above mentioned trees. Determinations of cellulose in this soil and in soil that was in cultivation five years or more were made using the method suggested by Charpentier (5) and modified by Barthel and Bengtsson (3) and very low amounts were found. Nitrates were always found in some quantity in this particular soil, but the rate of formation was retarded. Summing up all the evidence in our studies thus far it seems to indicate that these resinous substances in this particular soil are toxic to plant growth and may be a factor in retardation of nitrification during the early period of cultivation.

#### LITERATURE CITED

- (1) Alway, F. J. Agricultural value and reclamation of Minnesota peat soils. Univ. of Minnesota Agr. Expt., Sta. Bul. 118.
- (2) Anderson, J. A. 1926. The influence of available nitrogen on the fermentation of cellulose in the soil. *Ibid.* 21: 115.
- (3) Barthel, C., and Bengtsson, N. 1924. Action of stable manure in the decomposition of cellulose in tilled soil. *Soil Sci.* 18: 185.
- (4) Charpentier, C. A. G. 1920. Quantitative determination of the cellulose-decomposing power of the soils. *Meddel. Centralanst. Forsöksv. Jordbruksområdet* [Sweden], No. 205.
- (5) Gibbs, W. M., and Werkman, C. H. Effect of tree products on bacteriological activities in soil: I. Ammonification and nitrification. *Soil Sci.* 13: No. 4.
- (6) Koch, A. 1914. Über die Einwirkung des Laub- und Nadelwaldes auf den Boden und die inn bewohnenden pflanzen. *Centbl. Bakt. [etc.]. Abt. 2*, 41: 545.
- (7) Nemec, A. 1926. On the degree of humification of the dead covering of forest soils. *Proceedings of the Internatl. Soc. Soil Sci. New Series*, No. 3, 2: 255.
- (8) Tschirch. *Allen's Commercial Organic Analysis v. II*, part 3, p. 142.
- (9) Viljoen, J. A., and Fred, E. B. 1924. The effect of different kinds of wood and wood pulp cellulose on plant growth. *Soil Sci.* 18: 199.
- (10) U. S. Dept. Agr. Bur. Soils, Soil survey of Latah county, Idaho.

# FERTILITY STUDIES OF AN ABNORMAL IOWA SOIL ("PUSH" SOIL)

W. H. STEVENSON AND P. E. BROWN

*Iowa Agricultural Experiment Station, U. S. A.*

## INTRODUCTION

Areas of abnormal unproductive soil, known locally as "push" soils occur rather commonly in southwestern Iowa in the Southern Iowa Loess Soil Area. They are usually small in size, ranging from one-tenth of an acre to one or two acres in extent and they are found on hillsides, generally about halfway down the slopes.

These "push" soils have not been differentiated as a soil type because of their small extent and also because of the fact that they represent a very local soil condition rather than a characteristic soil formation. They have been mapped with the Shelby silt loam and their occurrence noted in the reports although it is well-known that they are quite different from the typical Shelby and they should be distinguished at least as a "shallow phase."

The name "push" soils has been popularly applied to these areas because of the fact that when they are plowed, the shallow surface soil is pushed aside and the plow point will not penetrate the heavy, impervious clay subsoil. The soil adheres to the plow in a sticky mass which must be removed by hand as the plow will not scour. Sometimes the soil "balls" up before the plow and it is forced out of the ground.

The areas are formed because of the washing away of the loessial covering on the hillsides and the consequent appearance of the heavy impervious Kansan drift clay near the surface. Sometimes this clay is exposed at the surface, the extremely active erosion, to which this land is subject, having led to the entire removal of the surface covering.

So-called "seepage" spots are usually found associated with "push" soils. They occur where the heavy clay subsoil appears at the surface. The rainfall does not penetrate the impervious subsoil but flows along under the surface soil over the clay layer, issuing from the soil where the clay is exposed further down the slope. The occurrence of these "seepage" spots makes drainage of special importance in the reclamation of "push" soil areas.

The surface soil of the "push" soil areas where any surface covering occurs, is a brown to dark-brown silt loam or loam. It may be of drift or loess origin but as most of the profile resembles the Shelby Series, the

soils are classified as Shelby, as has been noted. The subsoil is yellowish-brown to brown sticky, somewhat sandy clay, the lower subsoil being a bluish, sticky, gritty, impervious clay. The topography is rolling to sharply rolling.

Four samples were analyzed for total plant food content and the results are given as pounds per acre of two million pounds of surface soil and six million pounds of subsoil in Table 1.

*TABLE 1.—Plant food of "Push" soils, expressed as pounds per acre of two million pounds of surface soil and six million pounds of subsoil*

Samples	Total phosphorus	Total nitrogen	Total organic carbon	Limestone requirement
1. Surface	1,643	3,867	42,800	6,000
Subsoil	3,556	3,616	45,576	3,000
2. Surface	1,482	3,250	33,792	10,000
Subsoil	2,425	2,774	30,195	2,000
3. Surface	1,199	3,699	44,535	5,000
Subsoil	1,576	3,026	28,881	1,000
4. Surface	835	3,110	38,942	6,000
Subsoil	1,616	3,531	35,010	2,000
Averages				
Surface	1,289	3,481	40,017	6,750
Subsoil	2,293	3,236	34,915	2,000

The usual method of analyses were employed for the phosphorus, nitrogen and carbon and the Truog qualitative tests was used for determining the limestone requirement.

The results show that the soils are not high in phosphorus but they are fairly well supplied with nitrogen and organic carbon. They are all acid in reaction and show a rather considerable limestone requirement. The physical conditions of the soils are so poor that it would seem probable that there is an insufficient production of available plant food and hence applications of manure to stimulate bacterial action and improve the physical conditions would seem to be desirable, along with the addition of lime to remedy the acidity.

### TREATMENTS AND CULTURAL METHODS

These treatments, with drainage, deep tillage and subsoiling were employed in a series of field experiments which were laid out on a typical "push" soil area in 1914. Eight of the plots were one-tenth of an acre in size, 4 were one-twentieth of an acre and 2 about one-twenty-first of an acre. The area was drained by the use of tile laid around it and additional tile was laid between the drainage plots. Manure was added at the rate of 10 tons per acre except on Plot 2 where 12 tons were employed.

TABLE 2.—Field experiment "push soil", Union County, Creston Field

Plot No.	Treatment	1914 Wheat bu. per A.	1915 Corn bu. per A.	1916 Oats bu. per A.	1917 Height of soy- beans, in.	1918 Wheat bu. per A.	1919 Corn bu. per A.	1920 <sup>a</sup> Oats bu. per A.	1921 Clover tons per A.	1922 <sup>b</sup> Corn bu. per A.	1923 Oats bu. per A.	1924 Clover tons per A.	1925 Tim. and clover tons per A.	1926 Corn bu. per A.
1	Deep tillage	29.3	50.6	50.0	10.0	13.6	29.1		1.92	60.1	37.4	0.44	0.78	41.1
2	Do +manure	48.5	67.3	80.0	16.0	36.3	43.5		2.51	68.4	37.4	0.49	0.51	39.9
3	Check	17.4	45.6	35.0	7.5	6.8	20.1		0.57	31.5	18.4	0.17	0.14	17.3
4	Air-slaked lime	19.4	49.7	38.0	7.25	10.2	22.3		0.66	37.2	21.6	1.20	0.41	41.1
5	Manure+limestone	41.6	62.1	75.0	9.5	20.4	38.3		1.90	65.1	39.8	0.86	0.37	36.8
6	Limestone	16.6	42.2	42.0	8.0	11.3	28.1		1.38	45.6	31.8	0.56	0.23	24.5
7	Manure	34.9	45.3	76.0	9.5	21.5	19.6		1.77	51.5	33.0	0.57	0.34	35.2
8	Check	23.1	33.7	37.0	8.0	15.9	18.3		1.03	33.0	20.4	0.22	0.21	14.4
9	Drainage +manure+limestone	21.9	48.2	76.0	9.0	23.8	41.9		2.41	56.9	30.6	0.36	0.17	41.6
10	Do +manure	39.2	52.5	78.0	9.5	26.1	40.1		1.92	66.5	36.4	0.43	0.19	36.6
11	Do +limestone	16.3	44.1	44.6	7.5	15.9	37.3		1.69	37.7	25.0	0.20	0.11	29.8
12	Do	16.5	50.9	43.0	7.0	13.6	26.8		1.18	44.3	26.1	0.19	0.11	22.4
13	Subsoiled +manure									62.0	45.2	0.82	0.61	53.3
14	Do									52.7	29.6	0.77	0.53	45.3

<sup>a</sup> Results lost on account of a misunderstanding of telegrams. The oat crop was good and differences were apparent on all plots.

<sup>b</sup> Plots 13 and 14 were added in the fall of 1921.

Limestone was applied at the rate of 2 tons per acre and slaked lime in an equivalent amount. Deep tillage was accomplished by the use of a deep tillage machine and subsoiling was practiced on the plots added in 1921 by the use of a subsoiler attachment. The surface soil on the plots varied in depth but as the variation was typical of "push" soils, no attempt has been made to correlate the results with the depth of soil.

The crop yields secured on these plots for 12 years are given in Table 2, no results being secured in one season.

It is quite evident from the data given that the drainage of such areas is of prime importance in their reclamation. The addition of manure is of large value probably mainly because of the improved physical conditions and hence better bacterial action in the soils, which it brings about. Deep tillage is apparently of special value and the use of the subsoiler is also of importance. The use of lime seemed to have an effect in some cases but it did not always show large returns.

As a result of these tests certain recommendations have been formulated for the handling of "push" soils and when properly followed they are proving quite successful.

In draining such areas, tile should be laid around the spots and across the hill above the areas in a line at right angles to the slope of the hill. It should be laid on the impervious subsoil just below the surface soil but deep enough to escape frost. If the surface soil is very shallow the tile should be placed in the subsoil and blinded in with coarse cinders or broken stone. Branch lines or laterals should be laid through the spots.

The application of farm manure is of large value and 10 to 12 tons per acre should be used on well drained areas where deep tillage or subsoiling has been practiced.

The use of a deep tillage machine is quite worth while but as such machines are expensive subsoiling with the subsoiler attachment may prove more desirable. It is very important that the heavy impervious subsoil be opened up and that organic matter be added. Manure is preferable to other sources of organic matter because of the greater stimulation in biological activities which it engenders. It seems possible too that a phosphate fertilizer might be used to advantage on these areas and tests of acid phosphate are recommended. Lime additions will be of value when legumes are to be grown.

Areas of "push" soils which are now quite unproductive may be reclaimed and made highly productive by the use of the treatments recommended.



# EFFECT OF FOREST FIRES UPON THE COMPOSITION AND PRODUCTIVITY OF THE SOIL<sup>1</sup>

F. J. ALWAY AND C. O. ROST  
*University of Minnesota, U. S. A.*

## INTRODUCTION

Forest fires are of annual occurrence in Minnesota, and more than 4,000,000 acres have been burned over in the last 10 years, during which time the area in a single year has varied from 42,000 acres in 1919 to 1,245,000 in 1917. Within the past 40 years three forest fires, in addition to the usual loss of mature timber and young forest growth, have caused serious loss of life and extensive destruction of property. The Hinckley fire, in 1894, caused 418 deaths, and the Baudette fire, in 1910, about 30, while 432 men, women and children perished in the fires of October 12, 1918, when about 200,000 acres were burned over within less than 24 hours and over 5000 houses burned, including the town of Cloquet, 10 smaller towns and villages and parts of 5 others.

In recent years there has been considerable discussion (8) in this country as to the effect of forest fires upon the productivity of the soil. The prevailing opinion has been well expressed by Van Hise:

"The fires do not simply confine themselves to the timber, but they burn the humus in the soil itself. Frequently, after a great forest fire, and especially if the fires run over the same area two or three times, there is left of the soil, sand and the other minerals, but little or no organic material" (17, p. 238).

Mosier and Gustafson (1917) later expressed much the same opinion, referring to analyses by Snyder (6, p. 152).

The only analytical data bearing upon the subject appear to be those reported from Minnesota by Snyder about the time of the Hinckley fire. In 1892 and 1893, as chemist of the Minnesota Agricultural Experiment Station, with the assistance of farmers and of some students he secured soils from all parts of the state. He made detailed analyses of more than 200 of these, including 2 surface samples from sandy land near Staples and one from "gray clay" over red subsoil taken in a woods near Hinckley. One of the two from near Staples contained 0.12 per cent nitrogen and the other 0.04. The first was from land, originally a pine and oak clearing, that had been under cultivation for a short time, while the other was from near-by uncultivated land that had been cleared of the pine and oak trees.

<sup>1</sup> Published with the approval of the Director as Paper No. 684 of the Journal Series of the Minnesota Agri. Expt. Sta.

In discussing the analyses of these samples Snyder attributed the low amount of humus and nitrogen to the fact that the land had been burned over and then left uncovered and unprotected (12, p. 49). Shortly after the fire in 1894 the student, who the year before had taken the Hinckley sample, visited the same field again and took a sample for comparison with the first. The first contained 0.12 per cent nitrogen (10, p. 182) and the second only 0.03 per cent (13, p. 29). Three of these four analyses, which constitute the only analytical data on the subject, and to which Snyder



FIGURE 1.—Map of Minnesota showing location of forest dealt with

has since referred in several places in connection with the discussion of losses of nitrogen and organic matter from the soil (11, p. 135; 13, p. 29; 14, p. 111), were incidental to studies distinct from the effect of forest fires. Only the one sample was taken specifically for this purpose and that one not by Snyder himself.

After the fires of October 12, 1918, the question being at once raised as to whether the soil had been impaired for agricultural use, the senior author visited the burned-over areas (figs. 1 and 2), having with him the data from a study of the soil of virgin Minnesota forests made in 1916 and on which the analytical work had just been completed. This investigation has since been published in part (3, 5). A year later the junior

author visited some of the burned-over areas and collected samples of soil for analysis while 2 years later the authors together revisited some of these in order to observe the growth of grass and clover which had been sown in the spring following the fire, where the burning had been heaviest.

### THE FIRE OF OCTOBER 12, 1918

Both the character of this fire and the meteorological conditions preceding and accompanying it were such as to make it probable that in many places the soil was adversely affected to as great a degree as in any pre-

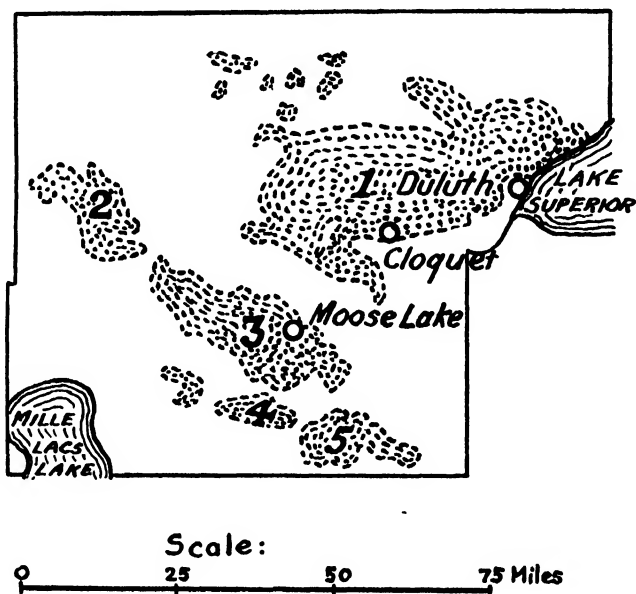


FIGURE 2.—A section of East-Central Minnesota showing areas burned over by the principal fires of October 12, 1918. 1—Cloquet-Duluth fire. 2—Bain fire. 3—Moose Lake fire. 4—Arthyde fire. 5—Bruno fire

vious fire, including the Baudette and Hinckley fires. All three occurred near the close of exceptionally dry seasons, the first on September 1, 1894, the second on October 7, 1910, and the last on October 12, 1918. Each was driven by a gale or hurricane.<sup>1</sup> That of 1918 had a wind with 50 to 60 mile rates, with a maximum speed of 76 miles. The Hinckley fire was described as "a cyclone of flame with a gale blowing 60 miles an hour," while in the Official Report of the Baudette fire it is stated that "it was a tornado that made the fire so fierce" (7, p. 18-19). All three are attributed to fires in bogs, which had been smouldering for days, or even weeks. The humidity of the air being very low the rising winds fanned

<sup>1</sup> U. S. Weather Bureau wind scale: gale—40 to 59 miles per hour, hurricane—60 miles or above.

these into flames, which, advancing before the gale, crossed hills and valleys, jumping plowed fields and small lakes that lay in their path.

For the city of Duluth, two residential districts of which were invaded by the fires during the afternoon of October 12, there is a meteorological record for the past 56 years. 1917 was a very dry year (Table 1) and in

TABLE 1.—*Precipitation before the fire at three U. S. Weather Bureau stations in the fire-swept district*

		Duluth	Cloquet	Moose Lake
		in.	in.	in.
Normal	Year	28.53	26.30	26.45 <sup>a</sup>
1916	Do	29.38	33.85	27.76
1917	Do	23.23	23.45	19.69
1918	January 1 to March 31	1.22	2.13	0.81
	April	2.02	1.49	1.47
	May	4.07	4.65	4.10
	June	0.84	1.40	0.30
	July	1.23	1.83	2.25
	August	2.32	2.27	3.30
	September 1-11	1.25	1.22	0.60
	Do 14	0.12	0	0
	Do 15	0	0.09	0
	Do 16	0	0	0.30
	Do 18	0.03	0.42	0
	Do 24	0.01	0	0.15
	October 4	0.07	0.12	0.09 <sup>a</sup>
	Do 5	0.17	0	0.01 <sup>a</sup>
	Do 6	0	0.03	0.01 <sup>a</sup>
	Do 7	0.11	0.15	0.27 <sup>a</sup>
	Do 8	0	0.02	0.03 <sup>a</sup>

<sup>a</sup> Data from Hinckley, 30 miles to the south; October record for Moose Lake lost in the fire.

1918 both the first 9 months and the 4 month period from June 1 to September 30, established new low precipitation records—13.61 inches and 5.80 inches, respectively. In the districts later swept by the fires, killing frosts occurred in most places during the first week of September, and before the end of the month temperatures of 20 to 22° F. had been recorded. September, although exceptionally cool, had an abundance of bright sunshine and was very dry, only 0.16 inch of rain falling in the last 19 days (Table 1). The first 11 days of October were almost rainless, but not exceptionally warm (Table 2).

The conditions on October 12, just before and during the fire, have been well described by H. W. Richardson, the observer of the U. S. Weather Bureau at Duluth:

"At the outset it must be borne in mind that this was not merely one great fire, but fifty to seventy-five or more, which, united to a considerable extent, were fanned to huge proportions by the wind, and then, with the increasing energy developed by the consequent violent air movement attending rapid combustion on such an enormous scale, advanced over vast areas with almost incredible speed. For some days before the great fires in question there had been numerous brush and peat-bog fires burning over limited patches. . . . Such fires are quite common to this section, especially during the dry periods in summer and autumn, and the public in general does not ordinarily regard them seriously. The conditions which favored the full development of the great fire were primarily those of drought (the season being the driest for 48 years) and the fresh winds that occurred on October 12.

TABLE 2.—Weather data for Duluth for first three weeks of October 1918

Day of month	Rainfall	Relative humidity <sup>a</sup>	Wind velocity		Sunshine	Character of day	Temperature	
			Average for 24 hr. <sup>c</sup>	Maximum for 5 min.			Mean	Departure <sup>d</sup> from normal
	in.	per cent			per cent			
1	0	68	19	39	0	Cloudy	48	-2
2	0	68	13	25	75	Partly cloudy	42	-8
3	0	62	15	22	99	Clear	46	-3
4	0.07	93	11	22	0	Cloudy	50	+1
5	0.17	100	7	20	0	Do	50	+1
6	0	94	13	23	97	Clear	44	-4
7	0.11	90	7	16	0	Cloudy	45	-3
8	0	71	14	31	90	Clear	56	+9
9	0	45	17	27	98	Do	58	+11
10	0	41	11	20	98	Do	62	+15
11	Trace	82	9	16	5	Cloudy	50	+4
12	0	42 <sup>b</sup>	32	65	58	Do	64	+18
13	0	62	18	48	46	Partly cloudy	48	+3
14	0	94	9	20	46	Clear	41	-4
15	0	76	7	17	66	Do	50	+5
16	0	100	17	29	28	Cloudy	48	+4
17	0	90	14	23	29	Do	46	+2
18	0	90	13	24	44	Partly cloudy	44	+1
19	0.14	89	12	21	0	Cloudy	45	+2
20	0	64	16	33	96	Clear	48	+5
21	0	61	7	19	94	Do	47	+5

<sup>a</sup> Averages based on 7 A.M. and 7 P.M. observations.

<sup>b</sup> The observations recorded on this day were 62 per cent at 7 A.M., 31 per cent at noon and 21 per cent at 7 P.M. Ordinarily the lowest relative humidity is around 3 P.M. or a little later and Richardson suggests that if readings had been made at that time on October 12 they might have reached low points between 15 and 20 per cent.

<sup>c</sup> The nearest whole number is used.

"Except for the continuance of the dry weather the general meteorological conditions on the morning of October 12 were not unusual for the season. . . . At Duluth the weather was clear until about 12:30 P.M., when smoke began moving in from westerly sources; but, because of the occasional previous occurrence of such a condition, the smoke attracted little attention. After 2:30 P.M. it increased considerably, the sun appearing red or being altogether obscured most of the time thereafter—a manifestation common to fires of unusual character. The wind gradually increased also, reaching 30- to 40-mile rates from the west at times until about 3 P.M. After that hour there was a steady rise to gale proportions, 50- to 60-mile rates from the west-southwest-northwest occurring between 4:15 and 9 P.M. After this hour the wind continued at 40-mile velocities until

about 2 A.M. of the 13th, subsiding materially thereafter. The highest 5-minute rate was 65 miles an hour from the west at 5:52 P.M., while the extreme speed for a less period was 76 miles about that time" (9).

On the morning of October 13 the wind had died down and the forest fires were simply smouldering. There were no further important advances of the fires, although no rain fell for 7 days and then only 0.14 inch at Duluth (Table 2), while the temperature continued near normal and the sunshine was abundant.

#### OBSERVATIONS ON THE BURNED AREAS IMMEDIATELY AFTER THE FIRE

The appearance of the burned-over parts of the forests to the northwest, west and southwest of Duluth, when visited on October 20 to 23,

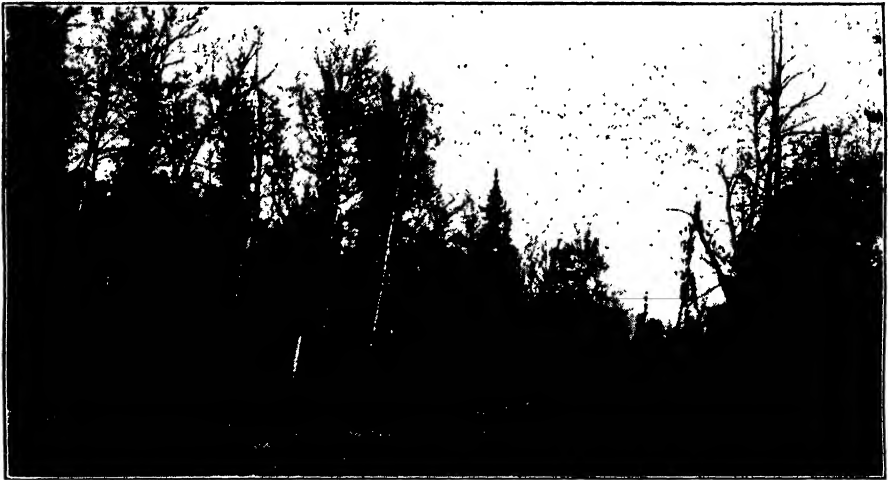


FIGURE 3.—Uninjured forest near Moose Lake, October 11, 1920

1918, was such as is usual right after a severe forest fire on mineral soil, and needs no detailed description (figs. 3 and 4). In the interval following the fire the only rain that had fallen, was 0.14 inch on the 19th (Table 2). The trees were dead, many partly consumed by the fire and many blown over and partly burned. Wherever there had been peat soil this had been deeply burned, the shallow roots of the trees exposed and more or less burned and the trees blown over, with the result that the woods in such places appeared as though a gigantic mower had just passed. This mowing down of the forest seemed limited to those areas that had had at least a shallow coating of peat, but in many places no trace of peat remained as evidence. In such places the gray or reddish peat ash covered the surface but elsewhere in the forest the ground was everywhere black from the charring, except where the roots of an over-

turned tree had brought up fresh subsoil. Over very extensive areas the forest floor had been entirely consumed, exposing the mineral soil. Even where the leafmold itself appeared to have been little affected the freshly fallen leaves that had been lying in it had been burned and the surface of the leafmold lightly charred. On the most deeply burned places, those where all the leafmold had been burned, and only a thin coating of char obscured the color of the mineral soil, there was no evidence of any baking or intense drying of the immediate surface, which everywhere, from the immediate surface to the depth to which it was examined, 4 or 5 inches, was distinctly moist, although less than 0.2 inch of rain had fallen (Table 2). That amount would be enough to penetrate air-dry soil of the prevailing texture to a depth of only about 1 inch.



FIGURE 4.—Typical burned-over forest, two years after the fire. October 11, 1920

Every bog seemed to have been set on fire and the better drained ones had been deeply burned. On many of the shallower bogs all the peat had been burned off and on the others the fires were still smouldering.

Part of the grass fields, meadows and pastures, had been badly scarred by the fires. These may be divided into three classes, according to the character of the surface before the fire, the first including those on mineral soil from which the stumps had been removed and the land plowed before seeding, while the second is made up of the stump pastures, in which the tree trunks and brush had been burned before seeding but in which many stumps still remained and in which the land had been seeded without plowing. A small number of meadows and pastures on peat form the last group and these had generally been burned even more severely than the uncleared bogs. In the stump pastures the surface had been scarred by burns (15, p. 21) especially around the stumps, the leafmold being burned off much as in the forest, but usually the deeply burned areas were small, from 3 to 10 feet in diameter and disconnected, while in the burned woods

there were solid tracts of 5, 10, or in places of even 40 acres where the leafmold remained on only a few square rods. The meadows and pastures on mineral soil that had been plowed before being seeded appeared no more affected than they would have been by an ordinary grass fire in

TABLE 3.—Location of fields and woods sampled, with character of forest growth

Reference No.	District	Range west	Township section north		Forest growth
			1. Uninjured forest		
21	Palisade	25	49	6	White pine, Norway pine, balsam, spruce, birch and hard maple.
22	Lawler	23	47	24	Originally white pine, at time occupied by aspen.
23	Automba	21	46	21	Balsam, spruce and birch with a few elm.
24	Do	21	46	19	Birch, elm, hard maple and balsam.
25	Lawler	22	46	19	Balsam, spruce, birch, oak, aspen and basswood with hard maple predominating.
26	Lawler	22	46	28	Balsam, birch, aspen with a few hard maple.
27	Do	22	46	26	Balsam and a few birch.
28	Kettle River	20	47	35	Balsam with a few birch and elm.
29	Moose Lake	20	47	14	Balsam and birch with a few elm, aspen and spruce.
			2. Fire-killed woods		
1	Automba	21	47	27	Aspen.
2	Kettle River	20	46	10	Balsam, basswood and birch.
3	Moose Lake	20	46	13	Balsam and birch.
4	Automba	21	47	27	Aspen.
5	Kettle River	20	46	10	Balsam, Basswood and birch.
6	Automba	22	47	25	Balsam, spruce, birch, basswood, ash, hard maple and small Norway pine.
7	Lawler	22	47	28	Spruce, birch, elm and aspen.
8	Palisade	25	49	6	White pine, Norway pine, balsam, spruce, birch and hard maple.
9	Kettle River	21	46	24	Balsam and spruce with a few aspen and birch.
10	Automba	21	46	22	Balsam, spruce and aspen with a few birch, hard maple, ash and elm.
30	Northeast Expt. Sta., Duluth	14	51	25	Balsam and birch.
31	Do	14	51	25	Do
			3. Burned pastures		
11	Moose Lake	20	46	24	Cleared
12	Do	20	46	23	Do
13	Kettle River	20	46	22	Do
14	Do	20	46	20	Do
15	Do	21	46	23	Do
16	Do	20	46	11	Do
17	Do	20	47	30	Do
18	Moose Lake	19	46	20	Do
19	Kettle River	21	46	20	Do
20	Do	21	46	12	Do
32	Northeast Expt. Sta., Duluth	14	51	25	Do
33	Do	14	51	25	Do

the spring or fall, and many of them were not burned over at all. On others the burning was limited to long, gradually narrowing tongues, that had shot out from the neighboring, burning woods on the west, southwest or northwest. Even on the most of these tongues the grass was still alive,



the injury being greatest right next the woods. The longest tongue observed did not exceed 40 rods.

There was comparatively little burning in the grain stubbles, the shocks having been removed before the fire. Here and there the stubble had been traversed by the fire, in whole or part, and in other places narrow tongues, like those in the grass fields, had extended into a field or even crossed it. In the case of the few fields that had been plowed shortly before the fire and the numerous small fields that had carried a cultivated crop, there was usually no evidence of the fire, the scattered dead weeds and corn stubble not being charred. Insofar as any possible injury to the soil was concerned it appeared that attention needed to be devoted only to the forests and stump pastures.

### COLLECTION OF SAMPLES FOR ANALYSIS

The sampled areas, 33 in all, fall into three groups, which will be referred to as *uninjured forest*—9, *fire-killed woods*—12, and *burned pastures*—12. The location of the fields and woods from which the samples were taken, together with the character of the original forest growth, so far as known, is given in Table 3. Typical views of the first two, taken 2 years after the fire, are shown in Figs. 3 and 4.

The sites in the uninjured forest sampled, although very close to burned areas, and in some cases standing like islands in the midst of these, had entirely escaped injury from the fires, not even the dry, freshly fallen leaves having been scorched. These areas were as nearly virgin as could be found in the burned over districts, and as a whole, probably more closely approached the conditions prevailing in the forest before the settlement of the district than those that were sampled as representative of the burned woods had done before the fire. Three samples were taken from each of these. The first represents the entire forest floor as defined by the Committee on Forest Terminology of the Society of American Foresters and the Canadian Society of Forest Engineers.<sup>1</sup> The second consisted of the immediate underlying 3 inches of mineral soil and the third of the 3-inch section below this. The three together would constitute approximately the portion of the surface that would be turned by the plow and mixed together if, immediately after felling the trees, the land were cleared without the use of fire and at once brought under cultivation.

<sup>1</sup> The term *forest floor* designates only the deposits of vegetable matter on the ground in a forest (4, p. 78). Three subdivisions of the forest floor were proposed by the committee.

1. Litter—the upper, but slightly decomposed portion of the forest floor.
2. Leafmold—the portion in which decomposition of the litter is so far advanced that its original form is not distinguishable (4, p. 82).
3. Duff—an intermediate layer of more or less decomposed organic matter between the litter and the leafmold.

In taking the samples of forest floor a 6 inch by 6 inch square was marked out by means of a long, sharp knife and a shallow trench dug at one side of this, so as to expose a vertical face of the square to a depth of 8 or 9 inches. After measuring the thickness of the forest floor the whole of it within the indicated square was transferred to a sack, care being taken to remove it as completely as possible from the surface of the mineral soil. Next, a block of the exposed mineral subsoil of the same area, 6 inches by 6 inches, and 3 inches in thickness, was transferred to a pail, well mixed and a sample saved. Lastly, the second 3-inch section was sampled in the same manner.

From each of the fire-killed woods and the stump pastures two sets of samples were taken, the one where the surface layer of organic residues had been entirely destroyed by the fire, only a layer of ash being left in its place, and the other close to this but where there had been little or no burning. The scorching with only a light char resulting, so commonly observed right after the fire, was not conspicuous, as a year had since elapsed. In none of the fields was it necessary to select these two related sites more than 2 feet apart. The closer together the two sets could be taken the greater was the probability that before the fire the soil on the two sites had been alike. On the unburned or lightly burned site a set of 3 samples were taken, as in the uninjured forest, while on the burned site only two were taken, the 1 to 3-inch section and the 4 to 6-inch section. In the case of the burned site, before removing the first section, the layer of ash was carefully scraped off with a knife and discarded.

### SURFACE LAYER BEFORE THE FIRE

The data on the forest floor of the uninjured forest and from the unburned sites in the fire-killed woods and on the leafmold in the burned pastures are reported in Table 4.<sup>1</sup> In thickness the forest floor in the burned woods was much like that in the uninjured forest, varying from 0.75 inch to 3 inches, with an average of 1.60 inch for the former and 1.44 inch for the latter. In the pastures it was somewhat thinner, ranging from 0.75 to 1.5 inch, with an average of 1.12, although in two pastures, Nos. 20 and 32, it was thicker than in half of the woods. In weight per unit area this surface layer was alike in the two groups of woods, averaging 27.2 tons per acre in one and 27.5 tons in the other. Computed from the individual samples it varied between 17 and 49 tons. In the burned pastures the weight of the surface layer, as sampled, was found to average 37 per cent higher than in the woods, which is to be attributed to more of the underlying mineral soil having been included in the samples. The tramping of the pasturing animals had compacted the leafmold, giving a much thinner but denser layer and this had become firmly bound by the grass roots to the mineral soil, increasing the difficulty of its separation.

<sup>1</sup> In the pastures under the term *leafmold* is included all that lay above the mineral soil.

This explanation is supported by the data on the volatile matter, which averages much higher in the surface layer of the forest and burned woods,

TABLE 4.—Amount and composition of leafmold in uninjured forest and in burned portions of fire-killed woods and pastures. Arranged in order of weight of nitrogen per acre

Field or woods No.	Leafmold			Volatile matter		Nitrogen		
	Thickness in.	Weight per sq. ft. lb.	Weight per acre tons	per cent	Weight per acre tons	In leafmould per cent	In volatile matter of leafmold per cent	Weight per acre lb.
1. Uninjured forest								
28	2.00	1.76	38.3	61.0	23.3	1.52	2.49	1163
23	1.25	1.32	28.9	75.1	21.7	1.84	2.45	1062
24	1.75	1.60	36.9	70.8	26.1	1.43	2.02	1056
25	2.00	1.31	28.5	59.4	16.9	1.63	2.74	928
26	1.50	1.26	27.5	58.9	16.2	1.49	2.53	820
29	1.25	0.77	16.7	70.9	11.8	1.94	2.73	648
21	1.25	1.28	27.7	50.4	13.9	1.08	2.14	627
27	1.25	0.77	16.7	43.1	7.2	1.79	4.01	600
22	0.75	1.10	23.9	33.3	7.4	.96	2.88	458
Average	1.44	1.25	27.2	58.1	16.1	1.52	2.67	818
2. Fire-killed woods								
10	1.50	1.73	37.7	65.6	24.7	1.79	2.73	1350
3	2.00	2.20	47.9	50.0	23.9	1.21	2.42	1158
4	1.75	1.31	28.5	47.7	13.6	1.73	3.63	987
1	1.25	1.15	25.1	71.2	17.9	1.84	2.58	923
30	1.50	1.19	25.9	69.8	18.1	1.54	2.21	799
7	1.25	1.30	28.2	47.5	13.4	1.36	2.87	768
2	3.00	1.21	26.3	63.4	16.7	1.40	2.21	736
9	1.25	1.06	23.0	51.2	11.8	1.55	3.03	714
5	1.25	1.19	25.8	58.4	15.1	1.28	2.19	662
8	1.25	1.28	27.6	50.4	14.6	1.08	2.14	627
6	1.25	0.95	20.6	43.8	9.0	1.32	3.01	544
31	1.00	0.60	13.1	69.7	9.1	1.74	2.49	455
Average	1.60	1.26	27.5	57.4	15.6	1.45	2.63	810
3. Burned pastures								
19	1.25	2.16	47.0	0	0	1.60	0	1505
32	1.50	1.59	35.1	73.8	25.9	1.79	2.42	1238
11	1.25	2.53	55.2	38.5	21.2	1.05	2.73	1160
20	1.50	1.54	33.5	55.7	18.7	1.70	3.05	1140
12	1.25	2.20	49.3	41.4	20.4	1.13	2.73	1114
33	1.00	1.43	31.1	61.8	19.2	1.63	2.63	1015
18	1.00	1.84	40.1	27.5	11.0	1.14	4.01	914
17	1.00	1.36	29.9	56.5	16.9	1.50	2.66	898
13	1.00	1.67	36.3	36.2	13.1	1.20	3.32	872
16	1.00	1.86	40.6	31.5	12.8	.94	2.98	765
15	0.75	1.17	25.4	41.0	10.4	1.38	3.36	700
14	1.00	1.23	26.3	37.2	9.5	.99	2.66	531
Average	1.12	1.72	37.5	45.6	16.3	1.34	2.96	988

with the result that with each of the three groups the average amount per acre of volatile matter is practically the same—16 tons.

The nitrogen content of the surface layer varied between extremes of 0.94 and 1.84 per cent, with averages of 1.52 per cent in the forest, 1.45 in the fire-killed woods and 1.34 in the pastures. The lower average found in the pastures is also to be attributed to the larger amount of mineral soil included, the weight per acre of nitrogen averaging 11 per cent higher than with either of the other groups. The percentage of nitrogen, computed to the volatile portion only of the surface layer, is in general appreciably the highest in the pastures.

It is evident that before the fire the three groups were very much alike in the weight per unit area of both organic matter and nitrogen, and that probably very little if any nitrogen had been lost from the surface layer between the time when the areas had been in virgin forest and that when as stump pastures they were sampled. The conversion of the stump pastures into plowed fields would not necessarily cause any immediate loss of nitrogen, but such a loss would soon follow. There probably had even been considerable gain in nitrogen in these pastures following their clearing, due to the growth of clover.

TABLE 5.—*Hydrogen ion concentration of surface layer from forest fire-killed woods and unburned parts of pastures. Arranged in order of values*

Uninjured forest		Fire-killed woods		Pastures	
Ref. No.	pH	Ref. No.	pH	Ref. No.	pH
22	5.2	7	5.3	12	5.7
24	4.8	5	4.9	15	5.7
27	4.5	10	4.8	17	5.7
23	4.1	31	4.8	13	5.1
25	4.0	1	4.6	18	4.9
21	3.7	2	4.5	16	4.7
28	3.6	3	4.2	33	4.6
29	3.6	4	4.1	11	4.3
		6	4.1	32	4.5
		30	3.9	14	3.9
		8	3.7	20	3.9
		9	3.2		

In acidity (Table 5) there is no great difference between the three groups. The pH values varied between 3.6 and 5.2 in the uninjured forest and between 3.2 and 5.3 in the fire-killed woods. Three of the pastures had somewhat higher values.

#### EFFECT OF THE FIRE UPON THE NITROGEN CONTENT OF THE SURFACE OF THE MINERAL SOIL

By the burning of the organic matter of the surface layer the nitrogen in it is driven off and lost to the soil. If the heat of the fire were sufficiently

intense and prolonged, much of the nitrogen in the surface of the underlying mineral soil would be driven off. From the data in Table 6 such a loss appears improbable. In the fire-killed woods the nitrogen in both sections averages only 0.02 per cent lower on the burned sites. In the pastures it is practically alike. The organic residues of the surface layer had been removed as completely by the fire as they could be removed by the careful use of a knife. Considering the very great range in the case of both 3-inch sections in both groups, the small differences found between the two sites are to be regarded as well within the range of experimental error.

TABLE 6.—Comparison of the nitrogen content of the surface 6 inches of mineral soil

Field	Unburned site			Burned site			Excess on unburned site		
	1-3-inch section per cent	4-6-inch section per cent	Average per cent	1-3-inch section per cent	4-6-inch section per cent	Average per cent	1-3-inch section per cent	4-6-inch section per cent	Average per cent
A. Fire-killed woods									
1	0.135	0.018	0.076	0.121	0.046	0.083	0.014	-0.028	-0.007
2	.081	.088	.084	.121	.059	.090	-.040	.029	-.006
3	.053	.041	.047	.094	.022	.058	-.041	.019	-.011
4	.145	.060	.102	.253	.056	.154	-.108	.004	-.052
5	.138	.094	.116	.117	.069	.093	.021	.025	.023
6	.058	.054	.056	.073	.053	.063	-.015	.001	-.007
7	.281	.105	.193	.219	.068	.143	.062	.037	.050
8	.048	.038	.043	.063	.058	.060	-.015	-.020	-.017
9	.259	.106	.182	.110	.069	.089	.149	.037	.093
10	.174	.091	.132	.197	.089	.143	-.023	.002	-.011
30	.149	.090	.119	.123	.176	.150	.026	-.086	-.031
31	.193	.136	.164	.203	.163	.183	-.010	-.027	-.019
Average	.143	.079	.111	.141	.077	.109	.002	.002	.002
Highest	.259	.136	.193	.253	.176	.183	.149	.086	.093
Lowest	.048	.018	.043	.063	.022	.063	.010	.002	.006
B. Burned pastures									
11	0.087	0.078	0.082	0.060	0.045	0.052	0.027	0.033	0.030
12	.109	.089	.099	.121	.088	.104	-.012	.001	-.005
13	.090	.056	.073	.078	.051	.064	.012	.005	.009
14	.070	.071	.070	.091	.070	.080	-.021	.001	-.010
15	.180	.041	.110	.114	.026	.070	.066	.015	.040
16	.058	.034	.046	.072	.051	.061	-.014	-.017	-.015
17	.167	.031	.099	.156	.052	.104	.011	-.021	-.005
18	.196	.117	.156	.189	.090	.139	.007	.020	.017
19	.130	.021	.075	.156	.040	.098	-.026	-.019	-.023
20	.205	.096	.150	.127	.032	.079	.078	.064	.071
32	.120	.129	.124	.119	.137	.128	.001	-.008	-.004
33	.153	.269	.211	.251	.196	.223	-.098	.073	-.012
Average	.130	.086	.108	.126	.073	.100	.004	.013	.008
Highest	.205	.269	.211	.251	.196	.223	.098	.064	.071
Lowest	.058	.021	.070	.060	.026	.052	.007	.001	.005

# EFFECT OF THE FIRE UPON THE MOISTURE EQUIVALENT OF THE MINERAL SOIL

One effect of heating soils hot enough to destroy the organic matter is to lower the moisture equivalent. If on the burned-off portions of the woods and pastures the fire had seriously raised the temperature of the surface soil the moisture equivalents of the samples from these would be lower. From Table 7 it will be seen that the differences between corresponding depths are too small and variable to indicate any change. The fractional

TABLE 7.—Comparison of moisture equivalents of surface 6 inches of mineral soil

Field	First 3-inch section			Second 3-inch section		
	Unburned site	Burned site	Apparent decrease	Unburned site	Burned site	Apparent decrease
A. Fire-killed woods						
1	24	22	2	16	17	-1
2	14	16	-2	15	14	1
3	12	14	-2	12	11	1
4	18	25	-7	12	15	-3
5	23	20	3	20	15	5
6	14	13	1	15	13	2
7	26	24	2	18	16	2
8	15	16	-1	12	15	-3
9	36	19	17	24	17	7
10	22	22	0	17	15	2
30	21	20	1	20	20	0
31	26	26	0	24	23	1
Average	21	20	1	17	16	1
B. Burned pastures						
11	21	21	0	19	22	-3
12	21	22	-1	22	20	2
13	17	16	1	15	14	1
14	19	21	-2	18	19	-1
15	21	16	5	15	11	4
16	16	18	-2	15	14	1
17	24	25	-1	16	14	2
18	23	22	1	23	20	3
19	21	23	-2	16	16	0
20	27	18	9	20	19	1
32	24	22	2	23	22	1
33	24	27	-3	19	23	4
Average	22	21	1	18	18	1

values are omitted as having no significance in such a comparison. If the data on No. 9, in the case of the fire-killed woods, are omitted the average difference becomes negligible, the value for the surface section from the burned sites being even a little higher.

### EFFECT OF FIRE UPON THE REACTION OF SURFACE SOIL

The pH values of the surface soil in both the fire-killed woods and the pastures are given in Table 8. In the 4-to 6-inch section no effect of the fire is shown but in the surface 3 inches the pH values have, in the majority of both groups, been raised a little. This may be attributed to the

*TABLE 8.—Comparison of pH values of surface mineral soil from unburned and burned sites in fire-killed woods and burned pastures. Fields arranged in order of values of surface section on unburned site.*

Reference No.	Unburned site		Burned site	
	1-3-inch section pH	4-6-inch section pH	1-3-inch section pH	4-6-inch section pH
A. Fire-killed woods				
2	5.2	4.4	6.5	4.5
7	4.6	4.4	4.7	4.5
10	4.3	5.1	4.4	4.5
8	4.2	4.2	4.2	4.3
1	4.2	4.5	4.5	4.2
4	4.1	4.4	4.1	4.2
6	4.1	4.2	4.6	4.3
30	4.0	4.3	4.4	4.4
31	4.0	4.2	3.8	4.2
3	4.0	4.3	4.2	4.3
5	3.5	3.9	3.7	3.8
B. Burned pastures				
19	5.3	5.9	0	0
12	4.7	4.5	5.2	4.5
16	4.6	4.3	4.2	4.3
18	4.6	4.4	4.6	4.6
15	4.5	4.4	4.9	4.5
17	0	0	4.1	4.5
13	4.2	4.3	4.2	4.2
14	4.2	4.2	4.1	4.3
33	4.1	4.5	4.2	4.2
11	4.1	4.1	3.9	3.8
32	3.9	3.9	4.1	4.3
20	3.9	4.3	4.2	4.1

lime and magnesia in the ash from the leafmold having reacted with the surface soil during the year that had elapsed between the occurrence of the fire and the collection of the samples.

In general it is safe to conclude that any immediate effect the fire had on the reaction of the mineral soil was favorable, but if the surface layer of organic residues had been simply mixed with the mineral soil and allowed to decay, the same amount of basic material would eventually have been released from the leafmold.

## EFFECT OF FIRE UPON THE PRODUCTIVITY OF THE SOIL

The samples of mineral soil taken from the uninjured forest (Table 9) are as similar in nitrogen content, reaction and moisture equivalent to those of the other groups as should be expected, considering that individual samples and not composites were employed. Thus there is no evidence that the fire caused any significant change in chemical composition or physical properties of the soil below the surface layer of organic residues, even where this was entirely burned off. The lime, phosphoric acid and potash of the leafmold would suffer no loss by burning and much would be left in a more immediately available form. The sulfur would probably suffer some loss through escape of sulfur dioxide but the forest floor carries very little sulfur. Whatever injury or benefit to the productivity of the soil has resulted from the fires is to be attributed to the destruction of this surface layer, and to this alone.

TABLE 9.—Composition and properties of surface mineral soil in the uninjured forest

Reference No.	Moisture equivalent			Nitrogen			pH value	
	1-3-inch section	4-6-inch section	Average	1-3-inch section	4-6-inch section	Average	1-3-inch section	4-6-inch section
				per cent	per cent	per cent		
21	14.6	11.8	13.2	0.048	0.038	0.043	4.2	4.2
22	14.3	8.7	11.5	.090	.044	.067	5.1	4.9
23	18.9	16.1	17.5	.122	.067	.094	4.5	4.6
24	25.0	17.3	21.1	.242	.105	.173	4.5	4.5
25	23.0	14.7	18.8	.229	.084	.156	4.7	4.7
26	28.6	21.0	24.8	.214	.091	.152	4.2	4.3
27	14.0	13.7	13.8	.080	.056	.068	4.0	4.4
28	16.6	12.0	14.3	.138	.042	.090	4.6	4.7
29	30.6	22.6	26.6	.436	.093	.264	3.8	4.3
Average	20.6	15.0	17.9	.178	.069	.123	0	0

The above being the case, the seriousness of the loss depends upon the amount and the availability of the nitrogen as it existed in the leafmold



and also upon whatever beneficial effect the leafmold would have upon the physical properties and biological relations of the surface soil, if it had been incorporated into this by plowing. On the burned areas in the present study the loss would vary from 455 to 1505 lb. of nitrogen and from 7 to 26 tons of organic (volatile) matter per acre, with an average of 872 lb. of nitrogen, and 16 tons of organic matter (Table 4). From the portions of the woods and pastures where the surface layer was not distinctly burned there would appear to have been no appreciable loss. So the loss on any particular tract can be estimated only after ascertaining what proportion of the surface shows distinct evidences of burning. With the 1918 forest fire the destruction of the surface layer was from place to place either practically complete or negligible. For some 40 acre tracts of



FIGURE 5.—A burned over woods five years after the fire. Near Bain, October 1923. In all parts of this wood the forest floor had been entirely burned off

mineral soil it reached 95 per cent (fig. 5) and on others fell as low as 10 per cent.

In the case of a severe forest fire near Grand Marais, Minnesota, in May, 1926, there was no evidence of the burning of any of the floor except the loose leaves, dead twigs and small branches. The examination of the burned-over area was made just after the rains had extinguished the fires. The trees, however, seemed burned almost as severely as by the fires of October 12, 1918 (fig. 6). The wet condition of the forest floor had protected it from serious injury.

#### STUDY OF FOREST FLOOR MADE BEFORE THE FIRES OF 1918

As the maximum injury which the forest fires can exert upon the mineral soils in a forest appears to be limited to the destruction of all the organic matter and the loss of the nitrogen carried in the forest floor one needs

only to know the amount of these originally present in order to be able to place the loss at so many pounds of nitrogen and so many tons of organic matter per acre. The organic matter in the floor is assumed to be closely enough indicated by the determination of the loss on ignition. Such an assumption would not be justified in the case of the soil. If we know also the content of nitrogen in the successive levels of the soil below the forest floor and assume that it would be no more valuable for agricultural crops than an equal amount of nitrogen contained in the floor, once the latter has been plowed under, we may estimate the proportionate loss of the total original amount of nitrogen in the soil and forest floor together.

The monetary equivalent of the loss in nitrogen and organic matter will depend upon the current values of cleared cultivated land of similar



FIGURE 6.—A week after the forest fire. Near Grand Marais, May 26, 1926. The forest floor in this was merely scorched

type in the same district. There is no justification for assuming that the nitrogen in the leafmold has the same value per unit weight as an equal amount of nitrogen in sodium nitrate or ammonium sulfate.

The 1916 study of the forest floor, mentioned above, consisted of a comparison of virgin forest soils with virgin prairie soils of the same age, both developed on the till plains of the Late Wisconsin glaciation (3, 5). Nine woods, the most nearly virgin that could be found, were sampled to a depth of 6 feet. Three near Mizpah (fig. 1) belonged to the spruce-balsam-birch type of forest, as did most of the woods sampled after the forest fire of 1918. The other six, near St. Paul and Taylors Falls, belonged to the maple-basswood type or form a transition from this to the oak-maple type. In collecting the samples of forest floor, in which were included, along with the more or less disintegrated plant debris, whatever fallen leaves and small woody fragments were present, extreme care was taken to separate the lowest part of the leafmold from the sur-

face of the underlying mineral soil, the first foot of which was sampled in 3-inch sections. The samples from each woods that were used for analysis were composites made up of equal amounts of 5 to 10 individual samples taken 10 to 15 yards apart in a straight line, the number depending upon the uniformity in thickness of the floor. The individual forest floor samples were each from 1 square foot and those of sections of the mineral soil were each from half a square foot. Thus the samples analyzed actually represent areas of 5 to 10 square feet in the case of the forest floor and half that area in the case of the underlying soil, instead of only a quarter of a square foot as with the 1919 samples. This difference in procedure would greatly reduce the effect of many local variations, so evident in Tables 6 and 7, that may be attributed to overturned trees and burrowing animals having thrown subsoil over the original surface.

The values (Table 10) are of the same general character as those found in 1919, the amount of nitrogen and volatile matter in the forest floor being much higher only in one of the woods, No. 3 at Mizpah.

TABLE 10.—Forest floor of nine Minnesota virgin woods

Group	Woods I	Woods II	Woods III	Average
A. Weight of dry forest floor, in tons per acre				
Hamel	25.0	27.8	12.6	21.8
Taylors Falls	20.2	20.9	19.6	20.2
Mizpah	30.3	23.3	96.7	50.1
B. Volatile matter in forest floor, in per cent				
Hamel	51.8	57.3	62.6	57.2
Taylors Falls	62.2	63.8	62.3	62.8
Mizpah	80.7	76.4	61.6	72.9
C. Weight of volatile matter in forest floor, in tons per acre				
Hamel	13.0	16.0	7.9	12.1
Taylors Falls	12.6	13.3	12.2	12.7
Mizpah	24.4	17.8	59.5	33.9
D. Nitrogen in forest floor, in per cent				
Hamel	1.53	1.57	1.76	1.62
Taylors Falls	1.86	1.74	1.82	1.81
Mizpah	1.89	1.55	1.47	1.64

## E. Nitrogen in volatile matter, in per cent

Hamel	2.95	2.74	2.81	2.83
Taylors Falls	2.99	2.73	2.92	2.87
Mizpah	2.34	2.02	2.39	2.25

## F. Weight of nitrogen in forest floor, in pounds per acre

Hamel	765	878	444	696
Taylors Falls	752	727	713	731
Mizpah	1,145	722	2,847	1,571

## G. Weight of nitrogen in surface 6 inches of soil, in pounds per acre

Hamel	2,823	2,730	2,319	2,624
Taylors Falls	2,104	2,265	2,141	2,170
Mizpah	913	613	828	785

## H. Weight of nitrogen in forest floor and surface 6 inches of soil together, in pounds per acre

Hamel	3,588	3,608	2,763	3,320
Taylors Falls	2,856	2,992	2,854	2,901
Mizpah	2,057	1,335	3,675	2,356

## I. Percentage of the total nitrogen reported in H above, that was carried in the forest floor

Hamel	21.3	24.3	16.1	20.6
Taylors Falls	26.3	24.3	25.0	25.2
Mizpah	55.6	54.1	77.5	62.4

In six of the woods the density of each of the four sections of the surface foot was determined (Table 11). In each woods the first 3-inch section was found much less dense than the second, which in turn was less dense than the third.

TABLE 11.—Density of different sections of surface foot in Minnesota virgin woods.  
Expressed as pounds per cubic foot

Depth inches	Taylors Falls			Hamel			Average of 6 woods
	Woods I	Woods II	Woods III	Woods I	Woods II	Woods III	
1-3	45	50	55	48	62	60	53
4-6	84	80	85	77	83	80	81
7-9	87	90	96	86	94	93	91
10-12	97	95	101	86	96	97	95

In texture the soils were much like those collected in 1919, as shown by the moisture equivalents (Table 12). The nitrogen content of the first 3-inch section of the surface foot was in all cases lower than that of the second 3-inch section (Table 13).

TABLE 12.—*Moisture equivalents of surface 6 inches of soil in Minnesota virgin woods*

Group	Depth	Woods I	Woods II	Woods III	Average
Mizpah	1-3	21	15	17	18
Do	4-6	15	13	18	15
Taylors Falls	1-3	23	25	25	24
Do	4-6	16	16	17	16
Hamel	1-3	30	28	25	28
Do	4-6	22	20	16	19

TABLE 13.—*Nitrogen in surface 6 inches of soil in Minnesota virgin woods*

Group	Depth	Woods I	Woods II	Woods III	Average
	in.	per cent	per cent	per cent	per cent
Mizpah	1-3	0.100	0.062	0.088	0.083
Do	4-6	.043	.031	.039	.038
Taylors Falls	1-3	0.288	0.317	0.250	0.285
Do	4-6	.076	.068	.071	.072
Hamel	1-3	0.335	0.293	0.267	0.298
Do	4-6	.129	.087	.079	.098

By employing the average values in Table 11 we may compute the amount of nitrogen in the surface 6 inches of the three groups that were sampled after the fires (Table 6 and 9), and by combining these values with those reported for the surface layer of organic matter in Table 4 obtain the relative amounts in the three levels, which mixed together would constitute the seedbed if the forest were converted into plowed land without the loss of any of the organic matter through fire (Table 14).

The proportion of the total amount of nitrogen in the second 3-inch section averaged practically alike in all, 20 per cent in the uninjured forest, 22 in the burned-over woods and 21 in the burned pastures. That in the leafmold averaged 45, 49 and 59 per cent, respectively. The extremes on the one side are shown by No. 16, a burned pasture, with 79 per cent in the leafmold and on the other by No. 22, a burned-over woods, and No. 29, uninjured forest, both with only 22 per cent in the surface layer.

#### VEGETATION EXPERIMENTS WITH LEAFMOLD

In order to determine the availability of the nitrogen in such leafmold as is found in the burned districts and also its beneficial or harmful effect

**TABLE 14.**—*Distribution of nitrogen between leafmold or forest floor and first 6 inches of mineral soil in woods, and pastures. The total amount in the three levels is placed at 100. Results expressed as per cent*

A. Uninjured forest													
	No. 21	No. 22	No. 23	No. 24	No. 25	No. 26	No. 27	No. 28	No. 29				Average
Forest Floor	66	61	52	38	44	40	58	54	22				45
1-3 inches	15	23	26	37	36	36	20	35	59				35
4-6 inches	19	16	22	25	20	24	22	11	19				20

B. Fire-killed woods													
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10	No. 30	No. 31	Average
Leafmold	50	48	78	70	41	60	35	66	37	53	41	22	49
1-3 inches	42	20	10	28	29	17	35	15	39	26	31	38	29
4-6 inches	8	32	12	2	30	23	30	19	24	21	28	40	22

C. Burned pastures													
	No. 11	No. 12	No. 13	No. 14	No. 15	No. 16	No. 17	No. 18	No. 20	No. 32	No. 33		Average
Leafmold	71	65	70	58	56	79	56	60	50	48	32		59
1-3 inches	12	15	16	16	33	11	34	21	29	20	17		20
4-6 inches	17	20	14	26	11	10	10	19	21	32	51		21

upon the growth of crops when it is mixed with the soil in large amounts, three vegetation experiments were conducted, using soils very poor in nitrogen (Table 15). That used in experiments A and B, was a silt loam from the fourth foot on University Farm at St. Paul, while that used in experiment C was from the surface 4 inches immediately below the leafmold in a spruce-balsam-birch forest near Moose Lake. In A and C the

**TABLE 15.**—*Vegetation experiments on the effect of leafmold on crop yields*

	Experiment A	Experiment B	Experiment C
Source of soil	University Farm	University Farm	Moose Lake
Depth of soil	Fourth foot	Fourth foot	Surface 4 inches
Texture	Silt loam	Silt loam	Fine sandy loam
Moisture equivalent	16.2	16.2	14.2
Nitrogen, per cent*	0.04	0.04	0.06
Source of leafmold	Moose Lake	University Farm	Moose Lake
Tree growth	Balsam-spruce-birch	Oak	Balsam-spruce-birch
Nitrogen, per cent	1.75	1.26	1.75
Reaction	Acid	Neutral	Acid
Date of beginning	1921	1921	1922
Number of successive crops	4	3	2
Crops	Barley Oats Red Clover	Barley Oats Red Clover	Oats Sorghum

leafmold used was from the same forest at Moose Lake, while in B it was taken from an oak grove on University Farm.

In Experiments A and B, started and carried on together in 1921, the same controls thus serving for both, the glazed earthenware pots used were 9 inches high and 8 inches in diameter, with an opening at the bottom, while in Experiment C, started in 1923, the jars were similar but of only half the capacity. In C the leafmold was mixed with the whole soil mass but in A and B with only the surface 6 inches. The pots were kept in a greenhouse and watered liberally with water from a deep well. The crops in each season were planted in February and harvested during

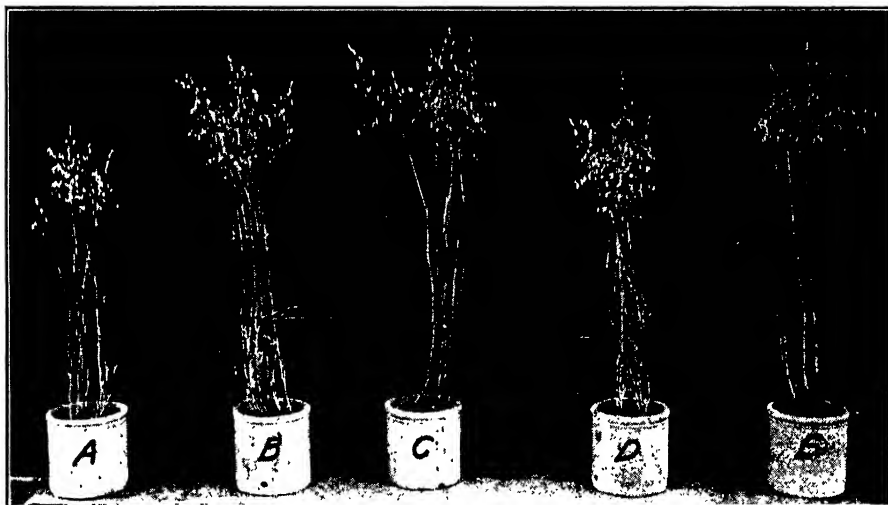


FIGURE 7.—Oats in Experiment C. All grown on surface 5 inches of mineral soil from Moose Lake uninjured forest. A—No application. Leafmold added to the others in amounts equivalent to following rates per acre: B—27.5 tons coarse, C—55 tons coarse, D—55 tons fine, E—110 tons fine

the following June, except with the clover, the first crop of this being cut in June and the second in August.

The leafmolds were used in two forms: (1) in a well divided but unground condition and (2) of the fineness of flour, obtained by using a ball mill on the dry material. In some cases half, and in others all of the leafmold, after being weighed out for the pots, was burned and the resulting ash mixed with the soil. No second application of leafmold or ash was made to any of the pots. As the average amount of leafmold found in the uninjured forest was between 27 and 28 tons per acre, amounts equivalent to 27.5 tons per acre, or multiples of this, 55 and 110 tons, were mixed with the soil. In experiments A and B six pots were left without addition of leafmold or ash to serve as controls, while in experiment C four were left as controls. The various applications of leaf-

mold and ash were represented by duplicate pots. Part of the pots in Experiment C, with the oats almost ripe, are shown in Fig. 7.

TABLE 16.—Yields of dry matter, on the control pots in experiments A, B and C

Crop	Experiments A and B							Experiment C*				
	1	2	3	4	5	6	Average	1	2	3	4	Average
	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams
Barley	0.97	1.30	0.60	1.40	1.50	1.20	1.16					
Oats	5.6	4.2	5.5	4.9	6.1	4.8	5.2	11.0	11.0		10.2	10.0
Clover, first cutting	10.6	12.7	13.8	12.6	11.7	12.3	12.3					
Clover, second cutting	9.1	8.8	9.2	12.0	4.3	7.2	8.4					
Sorghum	14.2	11.2	12.0	11.3	5.6	13.0	11.2	5.0		5.0	2.0	4.0

The actual yields on the controls are reported in Table 16 and the yields with the various treatments are expressed in relation to these (Tables 17, 18 and 19), the average of the yields on each set of controls being taken as 100. Both the leafmold and the ash increased the yield in all cases, no matter at which rate they were used, nor whether the leafmold was used in a ground or unground form. With the unburned material the yields increased with the rate of application, both in comparisons of the different rates of the course material and in similar comparisons of the finely ground product.

TABLE 17.—Effect of Moose Lake leafmold on the yield of crops on St. Paul subsoil. Experiment A. The yield on the jars without any application is placed at 100

Year	Crop	No leafmold	Leafmold, 27.5 tons per acre				55 tons per acre			Leafmold, 110 tons per acre	
			All burned	Half burned, half-coarse	Unburned		Half burned, half-coarse	Unburned		Unburned	
					Coarse	Ground		Coarse	Ground	Coarse	Ground
1921	Barley	100	289	252	300	347	426	501	452	880	819
1922	Oats	100	107	158	234	254	180	432	268	1104	298
1923	Red clover, first cutting	100	111	110	115	127	132	131	112	117	111
1923	Red clover, second cutting	100	129	148	114	118	166	132	133	115	136
	Average	100	159	167	191	211	226	299	241	554	341

Burning the whole or only half of the leafmold generally resulted in a lower yield than using it unburned. Grinding did not increase the beneficial effect, except slightly in the case of the lightest application in Experiment A. With the two heavier rates in that experiment and also in B, and with the only rate used in the comparison in C, the yields were higher when the leafmold was used without grinding. The leafmold



TABLE 18.—*Effect of St. Paul leafmold on the yield of crops on St. Paul subsoil. Experiment B. The yield on the jars without any application is placed at 100*

Year	Crop	No leafmold	Leafmold 27.5 tons per acre, coarse	Leafmold, 55 tons per acre	
				Coarse	Ground
1921	Barley	100	564	763	401
1922	Oats	100	226	429	252
1923	Red clover, first cutting	100	128	150	128
1923	Red clover, second cutting	100	163	170	132
	Average	100	270	378	228

from the oak grove at St. Paul caused somewhat higher yields than equal quantities of that from the balsam-spruce-birch forest near Moose Lake.

TABLE 19.—*Effect of Moose Lake leafmold on yield of crops on Moose Lake soil. Experiment C. The yield on the jars without any application is placed at 100*

Year	Crop	No. leafmold	Leafmold, 27.5 tons per acre		Leafmold, 55 tons per acre			Leafmold, 110 tons per acre	
			Half-burned half- unburned coarse	Unburned coarse	Half-burned half- unburned coarse	Unburned		Unburned	
						Coarse	Ground	Coarse	Ground
1923	Oats	100	168	185	121	230	277	251	197
1924	Sorghum	100	245	166	191	275	299	332	225
	Average	100	206	175	156	252	288	291	211

So it may be concluded that using up to 110 tons per acre of the leafmolds on the two soils, very poor in nitrogen, the effect found was in all cases beneficial, was not increased by grinding, and was lowered by burning.

## GROWTH OF GRASS FOLLOWING THE FIRES

In the spring of 1919 the Red Cross furnished settlers of the fire swept areas a mixture of timothy and alsike clover, which was seeded on the burned spots in the stump pastures and scattered broadcast among the fallen trees in the heavily burned woods, without any attempt being made to cover it. As a rule an excellent stand of grass was secured. Many of these seedings were examined in both 1919 and 1920 (fig. 8). In the burned woods in the fall of 1919 wherever the leafmold had been entirely burned off there was a good stand and both the timothy and the clover had made a vigorous growth, but where the leafmold had escaped injury or had been only charred on the surface there were only the native plants,

grasses, shrubs and weeds, the clover and timothy not being able to establish themselves. In the pastures the bluegrass and timothy on the unburned portions had grown as usual, while the new seeding had made an excellent stand on the burned spots and the fields were well covered with grass. In the fall of 1919 the growth of the timothy on the burned spots was usually so much ranker than on the older seedings, where these had escaped the fires, that the burning appeared to have increased the productivity of the soil, while a year later the clover was found to have



FIGURE 8.—Timothy in the burned over woods, October 11, 1920. It had been seeded in the spring following the fire of 1918

nearly all disappeared and the timothy in the burned woods had not made nearly as vigorous a growth that season as the older timothy on the nearby unburned fields. So in the second year it seemed the burning had reduced the productivity. This may be attributed to the loss of nitrogen. In the first season a lack of soil nitrogen would not so seriously affect the timothy as at that time the clover growing among it was able to furnish nitrogen for both itself and the timothy.

#### OBSERVATIONS ON DULUTH EXPERIMENTAL FARM

In the path of one of the fires of October 12, 1918, lay the Duluth Experimental Farm of the Minnesota Agricultural Experiment Station.

This had been established in 1913, when work was started in clearing off the trees of the spruce-balsam-birch type. All the standing timber, about 70 acres, was killed by the fire of 1918, when part of the farm build-

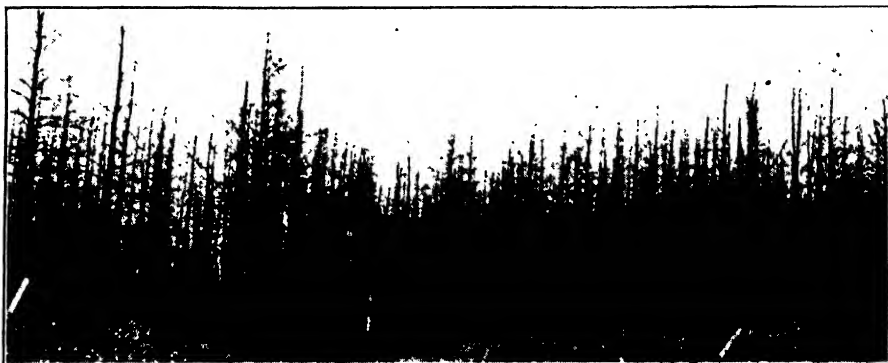


FIGURE 9.—Burned Jack pine woods, two years after the fire. Trees still nearly all standing

ings and livestock were burned, the men, women, and children being forced to take refuge in plowed fields and in the creeks under bridges (15, p. 19).

“The pastures were most severely injured where the stumps were thickest and the vegetable mold deepest, close in around each stump. Frequently the sod was so completely burned about and beneath the stumps as to produce the effect of elevating the



FIGURE 10.—A peat fire smouldering under a blanket of snow. Near St. Paul, December 28, 1923

stumps in midair. . . . In April, 1919, grass seed was sown throughout the timbered and stump area of the farm. A splendid stand was secured from a sowing of 2.5 to 5 pounds per acre of alsike and timothy mixed. Where the fire was most severe and the ash

deepest, better stands were secured, as the seed coming into direct contact with the moist clay underneath did not suffer for moisture, the ash affording excellent covering" (15, p. 20).

In the fall of 1919 a rectangle of 0.6 acre of heavily burned land, that had carried a heavy stand of timber, was stumped, broken and laid out in tenth acre plots and in the following spring planted to oats, barley and sunflowers. In 1921 a similar experiment was started on another part of the fire-killed woods. Both experiments were continued until 1924, comparing the yields with those on nearby land which had been broken and laid out in experiments before the fire. Thompson, who has been

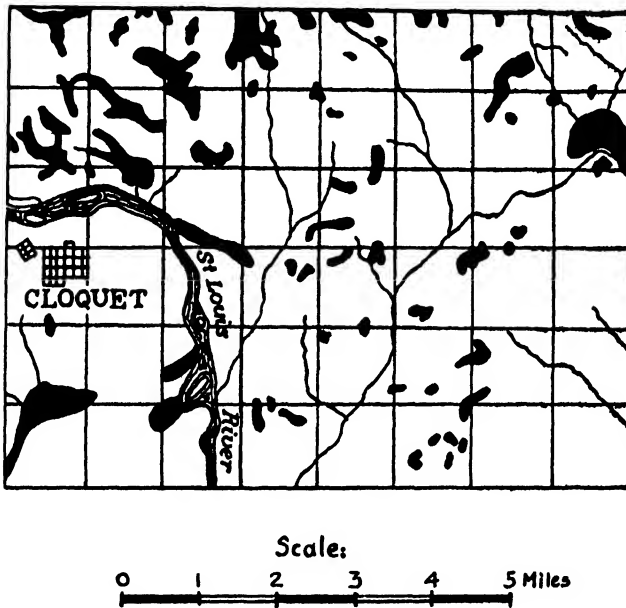


FIGURE 11.—Map of the vicinity of Cloquet, showing the distribution of peat

superintendent of the experimental farm since its establishment and was present during the fire, concludes that on the burned land potatoes and oats have yielded less, sunflowers more, and timothy-clover hay about the same as on the adjacent land that had been brought under cultivation before the fire (16, p. 20 and 23).

It should be pointed out that after the fire conditions on the experimental farm were not ideal for an experiment planned to determine the effect of burning, as while there was abundance of burned-over land there was no uninjured trace of timber left which could be cleared, plowed and tilled at the same time and in the same manner in order to show what yields would be obtained on land entirely untouched by fire but otherwise handled in just the same way. In April 1919 one of the authors in company with Thompson examined all parts of the burned-over woods

looking for a suitable site for such an experiment but without finding any that was satisfactory, the plan finally adopted seeming the best that circumstances permitted.

### FOREST FIRES ON SANDY SOILS

The fires of October 12, 1918 burned over very little forest on the light soils, upon which the Jack pine (*Pinus banksiana*) is ordinarily found and none of this was sampled, and none even examined until October 1920 when a burned woods near Moose Lake was visited. This had been burned while the fires were at their height, and every tree had been killed but the destruction of the forest floor was much less complete than in



FIGURE 12.—Burned over forest on peat near Baudette. On the part in the foreground the peat had burned deeply, allowing the trees to fall over

the nearby forest of the spruce-balsam-birch type. The appearance of the pines, nearly all of which were still standing seemed to indicate that the heat developed had been much less intense in the pine woods (fig. 9).

### PEAT SOILS IN FOREST FIRES

In dealing with the question of the effect of forest fires upon the productivity we must distinguish especially between mineral and peat soils. In many forests the latter are entirely absent but in the forests of northern Minnesota they occupy such a large proportion of the surface and are so widely scattered that a fire of any extent can scarcely occur without involving many bogs. They have played an important part in the three most serious fires, which were developed largely, if not entirely, from smoldering peat fires. The important rôle that the peat plays is due both to its very extensive distribution and the persistence of a fire once it has become established in it (fig. 10). Figure 11 shows the extent

and distribution of peat near Cloquet which was destroyed in the fire of 1918.

The effect of burning peat soils upon their productivity has previously been dealt with in detail by one of the authors (1, p. 104–117; 2, p. 106–113) and needs no discussion here. Typical views after a fire are shown in Figs. 12 and 13.

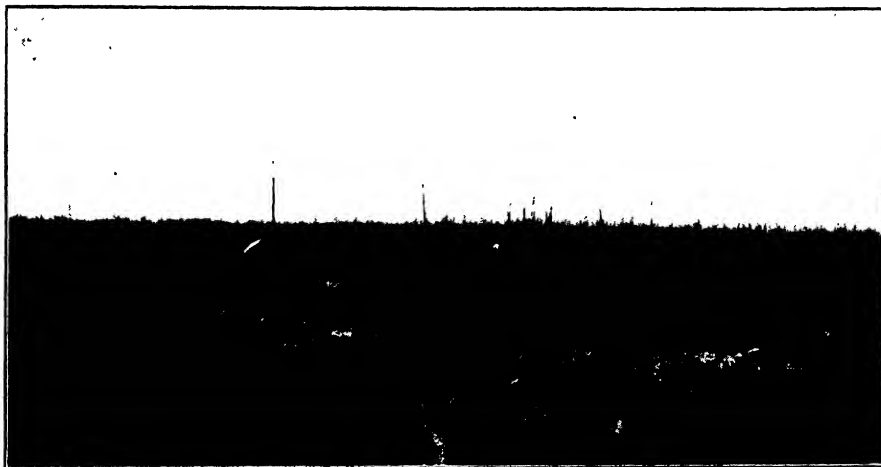


FIGURE 13.—View near Baudette, showing mounds of peat that had escaped the fire and boulders exposed by the burning of the peat cover. Here the trees had been dead before the fire which completely consumed them

#### LITERATURE CITED

- (1) Alway, F. J. 1920. Agricultural value and reclamation of Minnesota peat soils. Minn. Agr. Expt. Sta. Bul. 188.
- (2) ———. 1920. Report of Golden Valley Peat Experimental Fields, 1918 and 1919. Minn. Agr. Expt. Sta. Bul. 194.
- (3) ———. and Harmer, P. M. 1927. Minnesota glacial soil studies: II The forest floor on the late Wisconsin drift. Soil Sci. 23: 57.
- (4) Committee on terminology of the Society of American Foresters and of the Canadian Society of Forest Engineers 1917 Report on Forest Terminology. Jour. Forestry 15:68.
- (5) Harmer, P. M. 1927. Minnesota glacial soil studies: III Density of the surface foot in forest and prairie on the late Wisconsin drift. Soil Sci. 23: 73.
- (6) Mosier, J. G., and Gustafson, A. F. 1917. Soil Physics and Management. Philadelphia.
- (7) Lake States Forest Fire Conference, Official Report of 1910. St. Paul.
- (8) Lovejoy, P. S. 1921. The effect of forest fires upon the soil of the North Lake States. 22nd. Ann. Rept. Mich. Academy of Sci. p. 9.
- (9) Richardson, H. W. 1919. The northeastern Minnesota forest fires of October 12, 1918. Geographical Review, April, p. 220.
- (10) Snyder, H. 1894. The composition of native and cultivated soils and the effects of continuous cultivation upon their fertility. Minn. Agr. Expt. Sta. Bul. 30.
- (11) ———. 1895. Humus in its relation to soil fertility. Yearbook of U. S. D. A.

- (12) ———. 1896. Soils. Minn. Agr. Expt. Sta. Bul. 41.
- (13) ———. 1898. Effects of the rotation of crops upon the humus content and fertility of soils. Minn. Agr. Expt. Sta. Bul. 53.
- (14) ———. 1908. Soils and Fertilizers. Ed. 3. New York.
- (15) Thompson, M. J. 1921. Forced vs. Delayed systems of clearing stump land. Minn. Agr. Expt. Sta. Bul. 189.
- (16) ———. 1925. Effect of forest fires on land clearing and crop production. Minn. Agr. Expt. Sta. Bul. 200.
- (17) Van Hise, C. R. 1914. The Conservation of Natural Resources in the United States. New York.

# CHANGES IN SOILS LONG UNDER CULTIVATION

C. E. MILLAR

*Agricultural Experiment Station, Michigan State College, U. S. A.*

## INTRODUCTION

Much effort has been expended in determining methods of increasing the productivity of depleted soils by the use of lime, fertilizers, rotations and manures. Innumerable attempts have also been made to determine differences between soils of different degrees of productivity. So far as the writer is aware, however, little has been done to determine the actual changes a soil undergoes in passing from a productive to a depleted state, by making a comparative study of samples of the same soil type taken from depleted fields and adjoining areas of uncultivated land.

It is quite generally conceded that the supply of available nutrients and of organic matter decreases as a soil becomes depleted. In some cases the total amounts of phosphorus and of calcium are reported to have been reduced. Little information is available, however, regarding the relative amounts of soluble material such soils will give up when held at given moisture contents and temperatures for various periods, and probably of more importance, the *rate* at which soluble substances are liberated. The relative ability of depleted and virgin soils to renew the concentration of the soil solution when solutes are removed is of interest and also the role of organic matter in solution phenomena. Studies of the influence of such forces as steaming on the solubility of nutrients, and the amount and kind of replaceable bases are also of value.

It is the purpose of this report to summarize the results from investigations of some of these phenomena.

## REVIEW OF LITERATURE

Shedd (8) found the total phosphorus and that soluble in 0.2 N HNO<sub>3</sub> to be greater in 17 virgin soils than in the corresponding cropped samples.

As the result of a careful study of the phosphorus content of 9 soils which had been under cultivation for 40 to 60 years and of corresponding virgin soils Whitson and Stoddart (12) report large losses as the result of cropping with the exception of one case. Other soils which had received large amounts of manure during cultivation of 30 to 62 years showed large increases in phosphorus content in a number of cases, but more than half of these contained more phosphorus in the virgin than in the cropped and manured samples.



Shedd (9) divided the soils of Kentucky into areas on a geological basis and determined the total calcium and that soluble in 0.2 N  $\text{HNO}_3$  and in carbonated water in samples of cropped and virgin soils from each area. In the large majority of cases each method showed more calcium in the virgin than in the cropped soils.

Swanson and Miller (10) report the analyses of samples from long cropped fields and fields in native bluestem hay or buffalo grass pasture on several types of Kansas soils. In only two cases, however, were the cropped and virgin samples taken from areas in close proximity. The data for the two comparable soils show a decided loss in sulfur, nitrogen and carbon as a result of cropping. One shows a gain in phosphorus, one a loss of potassium and both a gain in calcium. An average of the data for all the soils shows a marked decrease in sulfur, nitrogen and carbon content and an increase in potassium and very little change in phosphorus and calcium. As a whole the results are rather unusual.

### COLLECTION OF SAMPLES

The samples in general represent the surface stratum 6 to 7 inches in depth. In some cases the sampling extended to the depth of the darker colored surface soil which in some virgin timbered soils was less than 6 inches while in some sandy cultivated soils it was more.

Two samples were taken from each soil. One from a field which had been under cultivation for a considerable period of years and had decreased more or less in productivity. The other was taken from a piece of woodland or from an old line fence row which was only a short distance from the site of the first sample. The first sample is designated as cropped or depleted while the latter is referred to as the virgin or uncropped soil. Thus the two samples represent the same soil type, the only difference between them being the changes resulting from the growth and partial removal of common farm crops, in one case, and from the growth without removal of more or less virgin vegetation in the other.

The samples were taken to the laboratory, air-dried and passed through a 2 mm. screen to remove pebbles and the coarser organic material. They were then stored in suitable containers until utilized in the laboratory experiments.

The soils studied were collected from 6 counties in Michigan and include 11 silt loams, 17 sandy loams, 5 loams, 13 loamy sands, and 2 clay loams. They represent the predominating soil types in the counties sampled.

### EXPERIMENTAL

#### RATE OF SOLUTION OF VIRGIN AND CROPPED SOILS

The first part of the work was devoted to the study of the rate of increase in concentration of the soil solution when the samples were maintained at

a uniform temperature with a water content slightly above saturation. It was decided to use the freezing point method of Bouyoucos and McCool (2) since this permitted rapid determination of the concentration of the soil solution directly in the soil without necessitating discarding the sample.

In some of the early experiments (4) the concentration of the soil solution of the virgin soils was generally much greater than that of the cropped soils after a ten-day period at 25° C. but at the expiration of thirty days the difference had largely disappeared. A decrease in moisture content tended to decrease the difference in rate of solubility and maintaining the samples at a low temperature both decreased the total solubility and the difference in solubility between the cropped and virgin soils. In later trials (6) it was found that washing on an ordinary filter until practically all the solutes were removed reduced to a large extent the tendency for material going into solution during the early part of the period to disappear from solution as the time of contact was prolonged. In consequence the following procedure was used for the major portion of the experiments.

About 60 g. of the air-dry sample was placed in an ordinary filter and washed with distilled water until practically free of soluble material as indicated by the freezing point method. After draining for an hour or so the soil was removed and thoroughly mixed by stirring in a tumbler. Samples were placed in freezing point tubes which were then stoppered and placed in a constant temperature chamber at 25° C. This procedure left the samples with a moisture content such that after settling, a column of water from an eighth to a quarter of an inch deep appeared above the soil. This condition was considered much more comparable for the different soil classes than the method of adding a given volume of water to a definite weight of soil.

The tubes were taken from the chamber every week or ten days during the period of the experiment, the stoppers removed and the contents thoroughly stirred to allow the escape of any gases which might have formed.

Freezing point determinations were made immediately after the samples were washed and placed in the tubes and at frequent intervals thereafter. The freezing point depressions for representative samples are presented in Table 1.

The data show that the virgin soils possess the power of giving up soluble material at a greater rate than the corresponding cropped soils. The divergence between the cropped and virgin samples in this respect is quite variable as would be expected since the degree of depletion in the different soils is quite different.

In most cases the greatest concentration obtained was considerably higher in the case of the virgin than of the cropped samples.

The tendency exhibited principally by the heavier soils to reach a

TABLE 1.—Rate of solution of virgin and cropped soils expressed as freezing-point depressions

Soil No.	Condition	Days maintained at 25° C.							
		0	1	2	6	10	20	40	60
Silt loams									
1	Cropped	0.000	0.005	0.005	0.010	0.010	0.020	0.043	0.023
	Virgin	.000	.009	.013	.020	.042	.053	.060	.041
2	Cropped	.001	.001	.009	.011	.012	.013	.026	.033
	Virgin	.004	.004	.010	.020	.040	.043	.074	.081
3	Cropped	.000	.009	.010	.013	.026	.029	.038	.017
	Virgin	.000	.009	.013	.024	.052	.064	.059	.045
Sandy loams									
4	Cropped	0.000	0.003	0.011	0.011	0.007	0.051	0.071	0.057
	Virgin	.000	.006	.013	.025	.030	.079	.070	.052
5	Cropped	.000	.005	.007	.012		.020	.045	.053
	Virgin	.000	.009	.019	.031		.062	.058	.069
6	Cropped	.000	.005	.007	.012	.018	.035	.033	.036
	Virgin	.000	.008	.011	.021	.030	.064	.071	.060
7	Cropped	.000	.003	.009	.008	.009	.021	.011	.014
	Virgin	.000	.010	.017	.029	.031	.054	.050	.049
Loamy sands									
30	Cropped	0.000	0.001	0.002	0.002	0.004	0.009	0.016	0.009
	Virgin	.000	.009	.018	.029	.039	.049	.054	.044
31	Cropped	.000	.001	.001	.001	.002	.001	.006	.001
	Virgin	.000	.011	.019	.035	.043	.063	.067	.054
10	Cropped	.000	.001	.002	.003	.015	.015	.010	.007
	Virgin	.000	.008	.013	.023	.030	.033	.026	.027
33	Cropped	.000	.001	.001	.001	.002	.002	.002	.002
	Virgin	.001	.002	.003	.010	.011	.013	.023	.012
Loams									
12	Cropped	0.000	0.002	0.008	0.014	0.020	0.029	0.035	0.037
	Virgin	.000	.002	.011	.020	.027	.028	.030	.033
13	Cropped	.000	.000	.001	.004	.011	.012	.027	.030
	Virgin	.000	.012	.015	.030	.040	.060	.059	.041
42	Cropped	.000	.007	.010	.018	.020	.030	.035	.020
	Virgin	.000	.011	.019	.033	.046	.055	.068	.059

maximum concentration of solution after 20 to 40 days and then decrease was undoubtedly due in part at least to the action of anaerobic organisms. It also seems highly probable that as hydrolysis and solution proceeded, changes may have occurred which resulted in precipitation of some of the dissolved salts. The possibility of adsorption should be considered.

## SOLUBILITY OF SUBSOILS FROM CROPPED AND VIRGIN AREAS

The finding of such a decrease in rate of solubility of surface soils as a result of continuous cropping at once suggests the question as to what effect depletion has on the solubility of the lower stratum or subsoil. To gain some information on this point a few subsoils were washed free of soluble salts, placed in freezing point tubes and maintained at 25° C. as outlined above. The freezing point depressions found at different times during the period of experiment are shown in Table 2.

TABLE 2.—Rate of solution of virgin and cropped subsoils expressed as freezing-point depressions

Soil class	Condition	Days maintained at 25° C.					
		0	1	5	20	40	60
		°C.	°C.	°C.	°C.	°C.	°C.
Very fine sandy loam	Cropped	0.000	0.001	0.002	0.002	0.002	0.000
	Virgin	.000	.002	.003	.002	.002	.000
Sandy loam	Cropped	.000	.000	.004	.006	.009	.006
	Virgin	.000	.003	.008	.012	.010	.007
Silt loam	Cropped	.000	.001	.003	.001	.002	.000
	Virgin	.000	.004	.006	.002	.003	.004
Silt loam	Cropped	.000	.000	.004	.007	.006	.007
	Virgin	.000	.000	.013	.020	.021	.018

It is surprising to note that these data show no appreciable difference in the rate of solubility of the subsoils from the depleted and virgin soils. In fact the solubility of all the samples is very slight. This agrees with the results of McCool and Millar (5) who determined the rate of solubility of subsoils collected from regions of great diversity of climatic conditions and found with few exceptions, a very low rate of solution.

RELATIVE ABILITY OF CROPPED AND VIRGIN SOILS TO RENEW THE  
CONCENTRATION OF THE SOLUTION AFTER REMOVAL OF SOLUBLE  
SALTS BY WASHING

Bouyoucos (1) found that soils maintained at 53° C. had a more rapid rate of solubility than at room temperature. A preliminary experiment by the writer showed that soils washed free of soluble salts and then maintained at 50° C. with a moisture content slightly above saturation develop a rather high concentration of solution after 40 hours. The virgin samples also showed a greater concentration than the corresponding cropped soils. It was deemed proper, therefore, to use this temperature and period of contact in the present experiment.

The soils were washed free of soluble salts on filters as previously described, then thoroughly mixed and quite large samples placed in freezing tubes. The tubes were stoppered and placed in the oven at 50° C. for 40 hours, after which the freezing-point depressions were determined and the soil again washed free of salts on small porcelain filters. The samples were made up to the proper moisture content and again placed in the oven for 40 hours. This procedure was repeated several times. The data are presented in Table 3.

TABLE 3.—*Relative ability of cropped and virgin soils to give up soluble material after successive washings to remove soluble salts, shown by freezing-point depressions*

Soil	Condition	First period	Second period	Third period	Fourth period	Fifth period
		°C.	°C.	°C.	°C.	°C.
Fine sandy loam	Cropped	0.011	0.008	0.010	0.008	0.007
	Virgin	.032	.016	.020	.016	.027
Silt loam	Cropped	.011	.002	.008	.004	.007
	Virgin	.028	.019	.025	.020	.017
Sandy loam	Cropped	.025	.013	.013	.013	
	Virgin	.030	.014	.021	.025	
Loamy sand	Cropped	.019	.001	.004	.002	.001
	Virgin	.044	.021	.022	.018	.020
Sandy loam	Cropped	.014	.003	.002	.003	.005
	Virgin	.027	.011	.011	.008	.013
Silt loam	Cropped	.025	.008	.008	.006	.013
	Virgin	.038	.018	.016	.014	.018
Loamy sand	Cropped	.007	.001	.000	.001	.002
	Virgin	.030	.013	.012	.010	.015

The results show that after the material going into solution during the first period is removed the depleted soils show only a feeble power to give up salts. It is interesting to note, however, that the concentration produced at each successive incubation is practically the same. This is in accord with the general observation that a soil will decline to a certain state of depletion and then continue to produce about the same yield from year to year. The productivity may, therefore, be taken as a measure of the rate of weathering of the less readily attacked minerals or the rate at which they give up soluble salts.

The virgin samples also show a decreased ability to give up soluble salts after the removal of the material liberated during the first period of incubation. As in the case of the depleted sample the solutions also attain approximately the same concentration at each succeeding incubation. The striking fact is that the concentration reached each time is much higher than is that of the corresponding depleted sample. This fact would lead one to predict the difference in productivity which exists.

### EFFECT OF REMOVAL OF ORGANIC MATTER BY OXIDATION WITH HYDROGEN PEROXIDE ON SOLUBILITY OF CROPPED AND VIRGIN SOILS

Peterson (7) found that oxidation of the organic matter of surface soils with hydrogen peroxide resulted in an increased amount of phosphorus, iron, and aluminum soluble in 0.2 *N* HNO<sub>3</sub> but not of calcium or manganese. Treatment of subsoils showed no increase in the solubility of phosphorus. His conclusion was that phosphorus, iron, and aluminum are held in organic complexes and that the mineral particles of the soil are not affected by the oxidation process. It was decided, therefore, to determine the effect of removal of the organic matter by this method on the rate of solubility in water of the soils under consideration.

The procedure followed was to place 60 g. of soil in an Erlenmeyer flask and add sufficient 3 per cent chemically pure peroxide to cover the soil. The contents of the flasks were thoroughly agitated every few hours and when the action had ceased enough 30 per cent peroxide was added to restore the liquid to approximately 3 per cent strength. When no further reaction was visible at room temperature the flasks were placed in a water bath at 30° C. and the above procedure repeated until practically all the organic matter was destroyed. The soils were now washed on filters with distilled water to free them of soluble materials after which they were thoroughly mixed and samples placed in freezing point tubes. These samples were maintained at 30° C. and freezing point determinations made after periods of 3, 7, 21 and 35 days. As controls, samples of the soils were given the same treatment as just outlined excepting that the peroxide was omitted. The data are found in Table 4.

TABLE 4.—*Effect of removal of organic matter by hydrogen peroxide on the rate of solution of cropped and virgin soils shown by freezing-point depressions*

Soils	Condi- tion	Days maintained at 30° C.									
		0		3		7		21		35	
		Normal	Treated	Normal	Treated	Normal	Treated	Normal	Treated	Normal	Treated
		°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.
Sandy loam	Cropped	0.000	0.000	0.000	0.005	0.004	0.017	0.005	0.035	0.014	0.034
	Virgin	.000	.008	.005	.006	.007	.023	.030	.031	.029	.031
Silt loam	Cropped	.000	.000	.018	.017	.022	.028	.044	.044	.043	.041
	Virgin	.002	.000	.020	.018	.026	.057	.049	.075	.038	.070
Fine sandy loam	Cropped	.003	.010	.018	.020	.019	.018	.034	.038	.037	.042
	Virgin	.005	.000	.018	.024	.022	.061	.046	.056	.040	.050
Loamy sand	Cropped	.000	.000	.000	.009	.003	.009	.017	.019	.018	0.19
	Virgin	.000	.003	.010	.018	.012	.019	.024	.025	.025	0.30

These results are very significant. In no case does the soil from which the organic matter has been removed show a lower rate of solubility than

the corresponding untreated sample. On the other hand three of the soils show a distinctly higher rate of solubility and others a tendency for greater solubility when the organic matter is removed. This would seem to indicate that the organic matter, in place of being either the source of soluble material or the liberating agent, in reality retards the liberation of soluble salts. This seems plausible since the so-called humus is known to form a coating around the mineral particles and this may act as a protecting agent. In fact microscopical examination of some of the soils before and after treatment with peroxide showed the mineral particles to be much lighter colored in the treated samples. It must be recognized, however, that the soluble salts given up before removal of the organic matter may come largely from the organic material while after the removal of the organic coating fresh surfaces of the mineral particles are exposed which have an even greater rate of solution. This is comparable to the finding of a decreased lime requirement of soils as the result of grinding.

The difference in rate of solubility between the cropped and virgin samples from which the organic matter had been removed was generally greater after the removal of the organic matter than before. This is very interesting inasmuch as it discredits the view occasionally expressed that soil depletion consists primarily in the loss of the major portion of the organic matter.

#### COMPOSITION OF MATERIAL GOING INTO SOLUTION FROM CROPPED AND VIRGIN SOILS

The next point of interest is to determine the differences in chemical composition of soluble material going into solution from cropped and virgin soils.

The procedure followed was to place 2000 g. of air-dry soil in an aspirator bottle and stopper with a one-hole rubber stopper over which a small pad of glass wool was placed as a filter. The bottle was then inverted and water added through the tubulation and allowed to percolate through the soil until most of the soluble salts were removed. The hole in the rubber stopper was then closed and enough water added to slightly more than cover the soil. After standing at room temperature for three weeks the plug was removed from the stopper and the solution allowed to drain out. The soil was then washed by adding successive portions of distilled water and allowing it to percolate through. Solution and washings were thoroughly mixed and aliquots used for analysis. The results in terms of parts per million of air-dry soil appear in Table 5.

These data bring out some interesting points. With one exception the depleted soils show a decidedly higher content of soluble sulfates. This is doubtless the result of the application of farmyard manure at various times during the period of cultivation. It would indicate that

on these soils at least sulfur has not as yet become a limiting element in plant growth.

The results for chlorine are somewhat more variable as four of the soils show larger quantities in the virgin samples, two in the cropped samples and no difference in the other case. The amount of soluble phosphorus is also so variable that it cannot be stated that a decrease in amount of water soluble phosphorus results from many years of cropping.

TABLE 5.—Material going into solution from cropped and virgin soils upon standing 21 days at room temperature with high water content

Soil	Condition	Alkalinity as $\text{Ca}(\text{HCO}_3)_2$	Cl	$\text{Fe}_2\text{O}_3$ $\text{Al}_2\text{O}_3$	Ca	Mg	$\text{SO}_4$	P
		p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.
Sandy loam	Cropped	109.35	12.59	2.95	6.75	2.21	13.66	1.08
	Virgin	341.70	18.89	8.02	17.75	6.75	5.03	0.80
Very fine sandy loam	Cropped	396.39	37.79	2.95	26.35	6.84	11.08	0.77
	Virgin	410.05	31.49	6.33	22.75	7.14	0.47	0.74
Silt loam	Cropped	177.65	6.30	18.14	11.00	3.23	12.67	0.71
	Virgin	628.75	6.30	30.80	43.00	7.30	6.08	0.74
Silt loam	Cropped	82.00	6.30	1.69	5.25	2.43	10.92	2.48
	Virgin	369.05	9.44	16.45	21.25	8.97	1.39	0.80
Loamy sand	Cropped	4.35	7.20	2.70	29.25	7.51	10.48	0.30
	Virgin	16.40	12.22	7.50	89.72	19.91	7.02	0.42
Sandy loam	Cropped	5.19	24.49	3.60	34.45	9.54	6.72	0.35
	Virgin	9.11	9.79	33.30	67.29	12.53	7.38	0.56
Loamy sand	Cropped	2.23	9.79	2.40	14.10	5.38	12.27	0.35
	Virgin	8.91	26.95	4.55	48.72	14.39	9.48	0.69

Of the remaining determinations including alkalinity, iron and aluminum, calcium and magnesium the results without exception show larger quantities going into solution from the virgin soils. The decrease in soluble alkaline earths might be predicted as a result of increased leaching due to cultivation. The decrease in soluble iron and aluminum, however, was scarcely to be expected inasmuch as it has been shown that an increase in soil acidity is often accompanied by an increase in solubility of these elements.

#### EFFECT OF STEAMING ON SOLUBLE MATERIAL IN VIRGIN AND CROPPED SOILS

König and Hasenbäumer (11) studied the influence of steaming on soils in an effort to correlate the amount of potash made soluble by this means with the necessity of adding potash fertilizers for the potato crop. In the soils studied the potash liberated by steaming 5 hours was equal to that removed by the potatoes. The conclusion was also reached that if 100 g. of soil yield 5 mg. of potash upon steaming potash fertilizers are



needed while if more than 8 mg. are liberated no additional potash is required for potatoes.

It would seem that steaming offers a means of measuring the amounts of material in cropped and virgin soils which could easily be made soluble.

TABLE 6.—Effect of steaming at 25 pounds pressure on the amount of soluble material in cropped and virgin soils

Soil No.	Condition	Freezing point depression		Soluble material		
		Before steaming °C.	After steaming °C.	Before steaming p.p.m.	After steaming p.p.m.	Increase p.p.m
Silt loams						
1	Cropped	0.006	0.029	150	725	575
	Virgin	.004	.045	100	1125	1025
2	Cropped	.017	.062	425	1550	1125
	Virgin	.038	.157	950	3925	2975
3	Cropped	.016	.061	400	1525	1125
	Virgin	.020	.073	500	1825	1325
Sandy loams						
4	Cropped	0.005	0.037	125	925	800
	Virgin	.008	.066	200	1650	1450
5	Cropped	.010	.049	250	1225	975
	Virgin	.019	.089	475	2225	1750
6	Cropped	.016	.058	400	1450	1050
	Virgin	.015	.069	375	1725	1350
7	Cropped	.010	.029	250	725	475
	Virgin	.017	.060	425	1500	1075
Loamy sands						
8	Cropped	0.013	0.038	325	950	625
	Virgin	.013	.056	325	1400	1075
9	Cropped	.010	.028	250	700	450
	Virgin	.013	.039	325	975	650
10	Cropped	.014	.020	350	500	150
	Virgin	.017	.053	425	1325	900
11	Cropped	.006	.027	150	675	525
	Virgin	.008	.039	200	975	775
Loams						
12	Cropped	0.011	0.053	275	1333	1058
	Virgin	.012	.060	300	1500	1200
13	Cropped	.011	.039	275	975	700
	Virgin	.021	.065	525	1625	1100

The procedure followed was to add 20 g. of soil to 14 cc. of distilled water in a freezing point tube. As pointed out by Bouyoucos (2) this proportion of soil and water will give complete saturation with enough surplus water to form a short column above the soil and is probably the best when determinations are to be made on several different soil classes. The prepared samples were allowed to stand a sufficient length of time to allow the readily soluble salts to go into solution and the freezing point determined. Each sample was then weighed after which they were submitted to steam pressure for two hours. After cooling the samples were re-weighed, the moisture content adjusted when necessary and the freezing point again determined. Trials showed that while considerable quantities of materials were made soluble by steaming at a pressure of 18 pounds much larger increases were obtained when a pressure of from 25 to 28 pounds was used. Accordingly steaming for two hours at this pressure was adopted as a standard procedure.

The freezing-point depressions of a few representative samples before and after steaming, together with the increases in soluble material are given in Table 6.

These data show that steaming puts large quantities of material into solution from all the soils tested and that virgin soils contain much more material rendered soluble by steaming than do soils long under cultivation. In general soils which give up large quantities of solubles upon standing in a saturated state of 25° C. also yield large amounts of soluble substances upon steaming. In most cases where steaming released a much larger amount of soluble material from the virgin than from the cropped sample the virgin soil also showed a much greater rate of solubility in water. However, there were a few exceptions.

#### NATURE OF MATERIAL MADE SOLUBLE BY STEAMING

The composition of the substances going into solution as a result of steaming was next determined. Five hundred g. of soil containing the same moisture content as was used in the preceding experiments, namely 20 g. of soil to 14 cc. of water, were placed in an Erlenmeyer flask and steamed for 2 hours at a pressure of from 25 to 28 lb. The soil was then filtered and washed several times. The combined filtrate and washings were analyzed by standard methods. The data are presented in Table 7.

These results show that in each case more basic material, as indicated by titration with standard acid, was liberated from the virgin than from the cropped soils. This correlates quite well with the results for calcium and magnesium except for the sandy loam soil in which case considerably more calcium and about the same amount of magnesium was liberated from the cropped than from the virgin sample. It should be noted in this connection that there is not a great deal of difference in the alkalinity of the two samples from this soil.

The results for Cl and  $\text{SO}_4$  are very irregular while with the exception of the sandy loam more  $\text{Fe}_2\text{O}_3 + \text{Al}_2\text{O}_3$  was liberated from the virgin than from the cropped samples. The same was true for  $\text{KCl} + \text{NaCl}$ . More P was also released from the virgin soils with the exception of one of the silt loams.

TABLE 7.—Material going into solution from cropped and virgin soils upon steaming 2 hours at a pressure of from 25 to 28 pounds

Soil	Condition	Alkalinity as Ca ( $\text{HCO}_3$ ) <sub>2</sub>	Cl	$\text{Fe}_2\text{O}_3$ + $\text{Al}_2\text{O}_3$	Ca	Mg	$\text{SO}_4$	P	KCl + NaCl
		p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.
Sandy loam	Cropped	228.8	19.5	30.0	69.9	19.14	77.0	4.25	8.5
	Virgin	255.1	7.1	28.5	54.1	20.39	59.7	4.55	7.5
Very fine sandy loam	Cropped	315.9	17.1	23.5	83.6	21.33	65.5	3.78	5.5
	Virgin	516.5	17.8	110.0	178.1	48.94	118.8	6.37	7.5
Silt loam	Cropped	218.5	7.1	29.5	102.9	23.68	103.4	3.57	1.75
	Virgin	443.5	13.8	42.0	128.5	24.66	79.8	5.31	7.5
Silt loam	Cropped	147.9	5.8	12.3	51.1	17.17	52.2	4.35	4.0
	Virgin	206.5	6.2	19.3	63.8	23.02	46.3	3.71	8.7

#### REPLACEABLE BASES IN CROPPED AND VIRGIN SOILS

The amounts and nature of the bases which may be replaced from a cropped soil as compared to those from a virgin sample should give valuable information regarding the changes a soil undergoes in becoming depleted. Work has been started along this line, using the methods of Kelley (3). While sufficient data has not as yet been accumulated to warrant its presentation it may be stated that indications point toward the liberation of larger quantities of Ca, Mg, Na, K, Fe and Al from the virgin than from the cropped samples.

#### LITERATURE CITED

- (1) Bouyoucos, G. J. 1919. Rate and extent of solubility of soils under different treatments and conditions. Mich. Agr. Col. Expt. Sta. Tech. Bul. 41.
- (2) ———, and McCool, M. M. 1915. The freezing point method as a new means of measuring the concentration of the soil solution directly in the soil. Mich. Agr. Col. Tech. Bul. 24.
- (3) Kelley, W. P. 1924. Replaceable bases in soils. Univ. of California Tech. Paper 15.
- (4) McCool, M. M., and Millar, C. E. 1918. Soluble salt content of soils and some factors affecting it. Mich. Agr. Col. Expt. Sta. Tech. Bul. 43.
- (5) ———, ———. 1920. The formation of soluble substances in soils taken from widely separated regions. Soil Sci., 10: 219.
- (6) Millar, C. E. 1919. The comparative rate of formation of soluble material in cropped and virgin soils as measured by the freezing point method. Ibid. 7: 253.
- (7) Peterson, P. P. 1911. Effect of heat and oxidation on the phosphorus of the soil. Univ. Wis. Agr. Expt. Sta. Res. Bul. 19.
- (8) Shedd, O. M. 1920. A short test for easily soluble phosphate in soils. Soil Sci., 2: 111.

- (9) ———. 1921. A comparison of the calcium content of some virgin and cultivated soils of Kentucky by an improved method for the estimation of this element. Kentucky Agr. Expt. Sta. Res. Bul. 236.
- (10) Swamson, C. O., and Miller, R. W. 1917. The sulfur content of some typical Kansas soils and the loss of sulfur due to cultivation. Soil Sci., 3: 139.
- (11) König, J., Hasenbäumer, J., and Schafers, J. 1923. Bezeichnungen Zwischen dem Nährstoffgehalts des Bodens und der Nährstoffaufnahme durch die Kartoffel. Landw. Jahrb. 58: 55.
- (12) Whitson, A. R., and Stoddart, C. W. 1909. Factors influencing the phosphate content of soils. Univ. Wis. Agr. Expt. Sta. Res. Bul. 2.

# DETECTION OF SULFUR-DEFICIENCY OF SOILS BY MEANS OF PLANTS

F. J. ALWAY

*University of Minnesota, U. S. A.*

## INTRODUCTION

The question of the possible sulfur-deficiency of soils received little attention until about 15 years ago, when Hart and Peterson (5) called attention to the erroneous views as to the amount of sulfur removed by various crops. Since then a considerable number of pot and field experiments have been conducted at different institutions in order to decide on what particular soil types sulfur fertilizers may be used to advantage. Gypsum and powdered sulfur have been the most commonly employed in the experiments because they are almost everywhere the most economical forms for practical field use. As the trial crop alfalfa has been the most frequently employed, with red clover second, while the use of rape, a very promising crop for the purpose, has been limited to plant house experiments.

Very striking increases in the yield of alfalfa and clover have been reported from several places, especially from parts of Oregon, Washington, and Idaho, but from present indications only a very small part of the soils of the country as a whole appear deficient in sulfur for even the most sulfur demanding crops. However, but a very small fraction of the cropped area of this country has as yet been subjected to satisfactory tests and the conduct of field trials for this purpose is a slow and expensive method of learning in which districts and on what soil types trials of sulfur fertilizers should be generally made on the individual farms. Up to the present no promising laboratory method has been proposed.

Having been studying the relation of the inadequacy of the phosphoric acid, potash and lime of soils to the content of these constituents in the crops grown upon them (1) a similar study suggested itself when marked evidence of extreme sulfur hunger suddenly developed on one of the Minnesota experimental fields. The results to date justify the expectation that by means of plant analyses it will be possible to greatly lessen the time and expense required to locate the districts where the trial of sulfur fertilizers should receive immediate attention.

## HISTORICAL REVIEW

Hart and Peterson (5, p. 3) found air-dry alfalfa hay to contain 0.29<sup>1</sup> per cent sulfur and air-dry red clover hay 0.16 per cent, which, on the

<sup>1</sup> In this article the percentages of sulfur in the crops are given to only the second decimal place. In the data quoted from other authors the third figure to the right of the decimal point is omitted.

oven-dry basis, are equivalent to 0.34 and 0.19 per cent, respectively. Using a Wisconsin surface soil in pot experiments, Hart and Tottingham (6) raised medium red clover and rape, both with and without the addition of sulfur fertilizers. With the clover there was no distinct effect on the sulfur content and no consistent increase in yield, but with the rape the sulfur content was doubled in one experiment and more than quadrupled in another although the yield was not increased. The applications were heavy, that of sulfur being at the rate of 550 lb. per acre, and those of gypsum and sodium sulfate at the rate of 1600 lb. Peterson (14) found that the addition of sodium sulfate and calcium sulfate to a Wisconsin soil greatly increased the sulfur content of clover, rape and radishes in pot experiments.

		per cent		per cent
Clover	Untreated	0.11	Sulfur treated	0.23
Rape	Do	0.18	Do	0.87
Radishes	Do	0.17	Do	0.82

Shedd (16) in pot experiments with 8 Kentucky soils found that the application of flowers of sulfur, at 100 and 200 lb. per acre, increased the content of sulfur in alfalfa, red clover and soy beans in all cases, and the 200 lb. application more than the 100 lb.; there was no distinct connection between the sulfur content of the crops grown on the control pots and the effect of the fertilizer upon either the yield or the increase in the sulfur content of the crops. The alfalfa from the unfertilized pots contained from 0.28 to 0.46 per cent sulfur and the red clover from 0.21 to 0.30 per cent. The increases in sulfur content due to the fertilization varied from 0.02 to 0.15 per cent in the case of the alfalfa and from 0.005 to 0.12 per cent with the clover (16, p. 97).

In field trials in Oregon, Reimer and Tatar (15, p. 33) found sulfur fertilizers to increase both the yield and the sulfur content of alfalfa. On the control and the fertilized plots, respectively, the sulfur content was 0.12 and 0.17 with Antelope clay adobe, 0.13 and 0.23 per cent with Tolo loam and 0.12 and 0.20 per cent on a barren coarse sand. On the first two soils sulfur was used at the rate of 300 lb. per acre, and on the third gypsum at 590 lb. per acre.

Miller reports field and pot experiments with Oregon soils. In field trials with alfalfa on 3 different soils (10, p. 98) the sulfur content was much increased.

		per cent		per cent
Soil 1.	Untreated	0.13	Sulfur treated	0.23
2.	Do	0.12	Do	0.17
3.	Do	0.12	Do	0.20

In pot experiments both the yield and the sulfur content of clover was increased. With rape the sulfur content but not the yield was increased. The sulfur content, determined on May 1 and again on June

1, was found to decrease with growth. In a later pot experiment Miller (11) found that the sulfur content of clover on one soil was raised from 0.20 per cent on the control to 0.26 to 0.41 per cent in the pots treated with sulfate while the yield was not affected. On 2 other soils, of which the sulfur content was 0.21 and 0.26 per cent on the controls, the sulfur content was not affected by the sulfur fertilization. On the first soil rape was sown following the clover and showed an increase in sulfur content of several hundred per cent.

Lomanitz (9), using Texas soils in pot experiments, found no increase in yield from sulfur applications but in general an increase in the sulfur content of alfalfa, which on the controls varied from 0.22 to 0.51 per cent.

Hall (4) analyzed 10 different lots of alfalfa hay, 5 secured from Kansas, 3 from Missouri and 2 from Illinois. The lowest sulfur content found was 0.37 per cent and the highest 0.56.

Bruce (3), using a Kansas soil, Oswego silt loam, in pot tests, found no increase in yield or in the sulfur content of alfalfa, which on the control was 0.44 per cent.

Neidig, McDole and Magnuson (12) in pot experiments with 6 Idaho soils found the sulfur content of alfalfa on the controls to vary from 0.14 to 0.30 per cent. Applications of sulfur at the rate of 100 lb. per acre raised the sulfur content of all, the increase ranging from 32 to 156 per cent. In the case of the 3 soils on which the sulfur application caused the greatest increase in yield the sulfur content of the crop from the controls was 0.14, 0.14 and 0.17 per cent, as shown by the data in Table 1.

TABLE 1.—Effect of sulfur fertilization upon alfalfa in pot experiments reported by Neidig, McDole and Magnuson

Soil	Moisture equivalent	Sulfur content of soil	Increase in crop yield from sulfur fertilization	Sulfur content of crop	
				From control	With 100 lb. per acre of sulfur
	per cent	per cent	per cent	per cent	per cent
Helmer silt loam	25	0.008	120	0.14	0.29
Palouse silt loam	26	0.023	91	0.17	0.43
Moscow loam	25	0.007	46	0.14	0.34
Sandpoint	40	0.016	35	0.23	0.39
Aberdeen	18	0.017	32	0.30	0.39
Boise silt loam	20	0.019	—3	0.27	0.43

Neller has studied the effect of sulfur fertilizers upon the sulfur content of alfalfa in the state of Washington, making use of both pot experiments and field trials (13). Using 3 soils, on only one of which sulfur applications caused distinct increases in yield, he found that on all 3 they raised

the sulfur content of the crop (Table 2). He reports a comparison of the sulfur content of alfalfa grown on the same type of soil, Ritzville silt loam, in both plant-house, 0.12 per cent, and field, 0.31 per cent. With an application of 500 lb. per acre of sulfur the corresponding values were

TABLE 2.—*Relation of sulfur content of alfalfa to crop increase on Washington soils.*  
From data of Neller

	Ritzville silt loam	Palouse silt loam
S content of soil, per cent	0.020	0.043
S content of crop from control pots, per cent	0.12	0.16
S content of crop with sulfur <sup>a</sup> , per cent	0.24	0.55
S content of crop with gypsum <sup>b</sup> , per cent	0.32	0.22
Crop on control pots, grams	31.8	41.7
Crop with sulfur, grams	61.7	37.4
Crop with gypsum, grams	53.4	41.4

<sup>a</sup> Sulfur at the rate of 500 lb. per acre.

<sup>b</sup> Gypsum at the rate of 1000 lb. per acre.

0.24 and 0.28 per cent, respectively. In pot experiments he tried applications of sulfur as high as 4000 lb. per acre. With the increasing rates of application the sulfur content of the crop increased but the yield was little affected by any of the applications except the heaviest, which depressed it.

	Sulfur application in pounds per acre				
	0	500	1000	2000	4000
Weight of crop, grams	25	32	33	29	9
Sulfur content, per cent	0.24	0.27	0.35	0.46	0.75

On irrigated Sagemoor fine sandy loam the alfalfa from the check plots contained 0.18 and 0.22 per cent sulfur and showed only a slight increase in yield from sulfur fertilization. On both of two adjacent plots given 500 lb. per acre of sulfur the alfalfa carried 0.29 per cent sulfur. On Ritzville fine sandy loam, which showed a slight crop increase from sulfur fertilization notwithstanding a shortage of water, the crop from the check plots carried 0.26 and 0.35 per cent sulfur and that from two adjacent plots, which had been given 500 lb. per acre of sulfur, carried 0.28 and 0.29 per cent.

Neller mentions that the alfalfa on the controls of Ritzville soil were of lighter green color and came into bloom later (13, p. 15).

Jones and Bullis (7) have recently published analyses showing the sulfur content of 60 samples of legumes from western Oregon. These were hand picked and afterwards very carefully handled to prevent shattering of leaves and small stems. The alfalfa showed a higher average sulfur



content and also a greater variation in sulfur content than the red clover, alsike, vetch or peas (Table 3).

TABLE 3.—Sulfur content of legume hay in western Oregon. From analyses by Jones and Bullis

Crop	Number of samples	Sulfur content		
		Lowest	Highest	Average
		per cent	per cent	per cent
Alfalfa	11	0.08	0.34	0.17
Red Clover	18	0.05	0.14	0.09
Alsike clover	8	0.07	0.14	0.11
Vetch	22	0.05	0.17	0.09
Peas	4	0.08	0.14	0.11

In Table 4 are summarized the data on the sulfur content of alfalfa reported by all the above mentioned authors, who used the modern methods for its determination. On the unfertilized crop grown in the field it varied from 0.08 per cent to 0.56. All the very low percentages reported are from Oregon, Washington and Idaho. The maxima re-

TABLE 4.—Sulfur content of alfalfa reported by different authors

Author	Source of samples	Sulfur content		
		Not treated with sulfur fertilizer		Treated with sulfur fertilizer
		Minimum (per cent)	Maximum (per cent)	Maximum (per cent)
A. Plants grown in the field				
Jones and Bullis (7)	Oregon	0.08	0.34	
Reimer and Tatar (15)	Oregon	0.12	0.13	0.23
Miller (10)	Oregon	0.12	0.13	0.23
Neller (13)	Washington	0.18	0.35	0.29
Hart and Peterson (5)	Wisconsin	0.34		
Hall (4)	Kans., Mo., Ill.	0.37	0.56	
B. Plants grown in pots				
Neller (13)	Washington	0.12	0.16	0.75
Neidig, McDole, Magnuson (12)	Idaho	0.14	0.30	0.43
Lomanitz (9)	Texas	0.22	0.51	0.62
Shedd (16)	Kentucky	0.28	0.46	0.53
Bruce (3)	Kansas	0.44		0.49

ported for the sulfur fertilized alfalfa from fields in the last two states are below those for the unfertilized crop from other states.

In the pot experiments the maximum is 0.75 per cent, reported from Washington, this being from an experiment in which an application of 4000 lb. per acre of sulfur had been used, so heavy that growth was greatly depressed.

### RESPONSE OF MINNESOTA SOILS TO SULFUR FERTILIZERS

An alfalfa experiment started on the Morris Experimental Farm in 1915 includes 4 plots to which gypsum was applied that season at the rate of 1000 lb. per acre. All the plots have been kept in alfalfa up to the present, the yield of every cutting being determined, but no distinct increase has resulted.

In the spring of 1921 gypsum was applied at the rate of 1000 lb. per acre to alfalfa at the substations at Waseca, Duluth, Grand Rapids, Crookston and Morris. At each place the field selected for the trial was in its second crop year or older. No effect of the treatment on the appearance of the crop being observed yields have not been determined.

On an experimental field at Hayfield, started in 1920, 2 groups of plots were laid out, A-B and C, the latter to be sown at once to red clover and the former to be seeded to alfalfa. Data from 16 of the plots in this field are employed in this paper:

#### GROUP C

- 2 plots—No application.
- 2 Do Sulfur, 200 lb. per acre.
- 2 Do Ground rock phosphate, 600 lb. per acre.
- 2 Do Ground rock phosphate, 600 lb. and sulfur, 200 lb. per acre.

#### GROUP A—B

- 2 plots—No application.
- 2 Do Gypsum, 1000 lb. per acre.
- 2 Do Hydrated lime, 3 tons per acre.
- 2 Do Hydrated lime, 3 tons, and gypsum, 1000 lb. per acre.

The applications were made and worked into the soil in April and the C group of plots seeded at once to red clover, with oats on the one-half of each plot and barley on the other. In 1921 2 crops of clover hay were cut. No effect of the sulfur was observed on either the cereals or the 2 crops of clover. In 1922 the plots were plowed and seeded to alfalfa. The A-B group was planted in 1920 to corn and in 1921 to alfalfa. The crop data from both groups are summarized in Part 7 of Table 6 in such a way as to show the effect of the gypsum on the yields. Placing the mean yield of the 8 plots given no sulfur fertilizer at 100 the mean for the 8 fertilized plots is found to be only 98.

The first systematic trials of the effect of gypsum on Minnesota soils are those included in experiments with alfalfa sown in the summer of 1922 on several new experimental fields, three on light sands, Bemidji, Backus and Cook Creek and the others on heavy soils, Voxland, Foss and Caledonia (Table 5). Three, Coon Creek, Backus and Bemidji, are respectively 18, 142 and 190 miles north and slightly northwest and the others, Voxland, Foss and Caledonia, are 46, 49 and 120 miles southeast of St. Paul. The field near Hayfield is 25 miles southeast of the Foss field. Those at Bemidji and Backus have light soils, Nymore loamy sand and were originally occupied by Jackpines (*Pinus banksiana*) while that at Coon Creek has an even lighter soil, Merrimac loamy fine sand (Table 5), and was covered chiefly by pin oaks (*Quercus ellipsoidalis*). The Caledonia field has a heavy soil, Tama silt loam, and had a natural growth mainly of red oaks (*Quercus rubra*) and scarlet oaks (*Quercus Cocinnea*). The Voxland, Hayfield and Foss fields were in prairie and have heavy soils, Marshall silt loam on the first and Carrington silty clay loam on the two others.

TABLE 5.—*Moisture equivalents of representative profiles on the experimental fields*

Depth feet	Bemidji Nymore loamy sand	Backus Nymore loamy sand	Coon Creek Merri- mac loamy fine sand	Voxland Marshall silt loam	Caledonia Tama silt loam	Foss Car- rington silty clay loam	Hayfield Carrington silty clay loam
1	6	8	4	26	27	29	27
2	4	6	3	26	27	26	24
3	3	4	3	24	28	20	20
4	3	4	2	23	28	19	18
5	3	4	2	22	27	19	18
6	3	4	2	20	26	20	18

On the 3 sandy fields and the Voxland and Foss fields the fortieth-acre plots were laid out and the applications made late in the fall of 1921 and the alfalfa sown the following June. On each of the 5 there were included: 3 or more plots given no application, 3 given 1000 lb. per acre of gypsum; 3, ground limestone (3 tons per acre on the light soils and 4 tons on the heavy) 3, limestone, phosphate and potash, and 3, limestone, phosphate and potash with gypsum. The applications of potash and phosphate were repeated in 1924 and 1926. The former was in the form of muriate and the latter in that of treble superphosphate, 200 lb. per acre in 1924 and 100 lb. in 1924 and again in 1926. This carried only 1.06 per cent sulfur, making the total amount added with the phosphate about 4 lb. per acre.

On the Caledonia field a crop of oats was plowed under about the middle of July, 1922, the various applications made, including none of gypsum alone, and alfalfa sown on July 25. This field gave only one cutting in 1923 but in each of the following seasons has given two.

A good stand remains from the original seedings on all the fields except that at Backus on which it was largely killed during the winter of 1925 to 1926. Each season the yield of every cutting of hay has been determined on each field.

From Table 6, in which the data reported are the averages for triplicate plots unless otherwise indicated, it will be seen that only at Bemidji and

TABLE 6.—Effect of gypsum upon relative yields<sup>a</sup> of alfalfa on different Minnesota experimental fields

		No lime		Lime		Lime, phosphate, potash	
Year	Number of cuttings	Yield without gypsum (tons)	Relative yield with gypsum (per cent)	Yield without gypsum (tons)	Relative yield with gypsum (per cent)	Yield without gypsum (tons)	Relative yield with gypsum (per cent)
1. Bemidji							
1923	1	0.81	135	1.02	127	1.08	108
1924	2	1.18	169	1.20	186	1.99	126
1925	2	1.67	160	1.65	176	2.11	154
1926	2	0.84	257	0.87	285	1.61	162
Total	7	4.48	177	4.74	188	6.69	144
2. Backus							
1923	1	0.69	88	1.48	97	1.29	119
1924	2	2.06	113	2.76	113	2.74	128
1925	2	2.03	133	2.53	128	2.65	132
Total	5	4.78	118	6.77	115	6.68	125
3. Coon Creek							
1923	2	0.95 <sup>b</sup>	94	1.58 <sup>c</sup>	102	1.78	97
1924	2	1.89	98	2.15	93	2.27	100
1925	2	1.94	107	2.26	105	2.32	108
1926	2	1.30	104	1.47	93	1.21	107
Total	8	6.08	101	7.46	100	7.58	103

<sup>a</sup> The yield on the plots receiving neither gypsum nor sulfur=100.

<sup>b</sup> Average of 17 control plots.

<sup>c</sup> Average of 6 limed plots.

TABLE 6 (Continued).—Effect of gypsum upon relative yields<sup>a</sup> of alfalfa on different Minnesota experimental fields

		No lime		Lime		Lime, phosphate, potash	
Year	Number of cuttings	Yield without gypsum (tons)	Relative yield with gypsum (per cent)	Yield without gypsum (tons)	Relative yield with gypsum (per cent)	Yield without gypsum (tons)	Relative yield with gypsum (per cent)
4. Voxland							
1923	2	3.32	102	4.21	99	3.85	112
1924	2	4.94	104	5.65	98	5.23	106
1925	2	5.89	103	6.29	102	6.61	111
1926	2	4.47	103	4.59	96	4.65	100
Total	8	18.62	103	20.74	99	20.34	107
5. Foss							
1923	1	0.76	72	1.16	107	1.37	106
1924	2	3.56	84	4.00	87	4.23	102
1925	2	4.06	99	4.24	103	4.66	110
1926	2	3.27	97	3.31	103	3.74	100
Total	7	11.65	92	12.71	99	14.00	104
6. Caledonia							
1923	1	<sup>a</sup>		0.69	77	0.35	170
1924	2			2.99	90	3.55	101
1925	2			4.24	111	4.79	101
1926	2			4.60	100	5.01	101
Total	7			12.52	100	13.68	103
7. Hayfield							
1922	2	3.33	93	3.81	101	<sup>b</sup>	<sup>b</sup>
1923	2	2.39	102	2.63	118	1.89	98
1924	2	2.82	88	3.41	89	3.38	105
1925	2	3.12	94	3.91	90	3.83	100
1926	2	2.64	95	3.08	92	3.58	109
Total	10	14.30	90	16.84	98	12.58 <sup>c</sup>	105

<sup>a</sup> No plots treated with only gypsum.<sup>b</sup> Alfalfa seeded in 1922. Four plots had received 200 lb. per acre of sulfur flour in 1920, and the other four neither sulfur nor gypsum.<sup>c</sup> For 4 years only.

Backus has any evidence been found of a response to gypsum and at the former it is far the more marked. If the mean of the yields on all the plots given no gypsum is placed at 100, that for all the gypsum treated plots becomes 170 at Bemidji and 119 at Backus. At Coon Creek it is 100, on the Voxland field 103, on the Foss field 98, at Caledonia 100 and at Hayfield 97.

The gypsum, where applied without lime, exerted a depressing effect on the growth of the alfalfa during the season of seeding, 1922, except on the Hayfield and Voxland fields, where no effect was observed. In the later seasons it has not noticeably affected the appearance of the alfalfa on any of the fields except those at Bemidji and Backus.

TABLE 7.—Yields on individual plots of Bemidji field showing effect of gypsum

Plot	Application	Yields per acre of hay				Total for 4 years
		1923	1924	1925	1926	
		tons	tons	tons	tons	tons
1	None	0.81	1.39	1.84	1.06	5.10
2	Do	.73	.98	1.61	.66	3.98
3	Do	.89	1.19	1.57	.79	4.44
	Average	.81	1.18	1.67	.84	4.48
1	Gypsum	1.01	2.13	2.56	2.32	8.02
2	Do	1.01	1.95	2.68	<sup>b</sup>	
3	Do	1.25	1.90	2.79	2.00	7.94
	Average	1.09	1.99	2.68	2.16	7.92
1	Marl <sup>a</sup>	1.01	1.36	1.83	1.16	5.36
2	Do	.81	1.06	1.45	.75	4.07
3	Do	1.25	1.17	1.67	.69	4.78
	Average	1.02	1.20	1.65	.87	4.74
1	Limestone	1.13	1.48	2.13	1.50	6.24
2	Do	1.01	1.16	2.09	1.37	5.63
3	Do	1.37	1.79	2.05	.99	6.20
	Average	1.17	1.48	2.09	1.29	6.03
1	Limestone and gypsum	1.09	2.29	2.79	2.45	8.62
2	Do	1.25	2.22	3.00	2.80	9.27
3	Do	1.57	2.19	2.92	2.20	8.88
	Average	1.30	2.23	2.90	2.48	8.91
1	Limestone, phosphate and potash	1.09	2.21	1.96	1.66	6.92
2	Do	1.01	1.97	2.01	1.58	6.57
3	Do	1.13	1.80	2.35	1.59	6.87
	Average	1.08	1.99	2.11	1.61	6.79
1	Limestone, phosphate, potash and gypsum	1.13	2.62	3.38	2.78	9.91
2	Do	1.09	2.36	3.20	2.78	9.43
3	Do	1.29	2.54	3.14	2.55	9.52
	Average	1.17	2.50	3.24	2.71	9.62

<sup>a</sup> 4 tons per acre carrying 92 per cent carbonates.

<sup>b</sup> Datum incomplete.

## RESPONSE OF BEMIDJI FIELDS TO DIFFERENT SULFUR FERTILIZERS

The effect of gypsum on alfalfa on the Main Field at Bemidji is evident from Part 1 of Table 6. From Table 7 it will be seen that this marked increase in crop yield was shown by every plot treated with gypsum. In 1923 there was no marked difference in appearance between the untreated and the gypsum treated plots at Bemidji and with the limed plots the yields were only slightly higher on those that had received gypsum. In 1924 at the time of the first cutting, however, all the gypsum treated plots stood out very distinctly, the alfalfa being taller and darker green in color.

In order to definitely decide whether the beneficial effect of the gypsum is due to its sulfur content many of the plots in the same field that previously had received no gypsum were treated late in October of 1924, or early in the following April, with 500 lb. per acre. In an adjacent field, referred to as the North Field, marled and seeded to alfalfa in 1923, plots were laid out and applications made of sulfur flour, sodium sulfate, magnesium sulfate, gypsum, and sodium chloride. Between the first and second cuttings in 1925 still other plots were treated, some receiving applications of 25, 50, 100 and 200 lb. per acre of sulfur and others 50, 100 and 200 lb. per acre of gypsum. In the spring of 1926 ammonium sulfate and sodium nitrate were applied to previously unfertilized plots.

The effect of all the sulfate carriers was similarly beneficial (Table 8) and that of the sodium nitrate almost negligible, making it clear that the sulfur in the gypsum was the cause of the increased yields. There is no doubt as to the extreme sulfur hungriness of the soil of the Bemidji field toward alfalfa.

At Backus the beneficial effect of the gypsum did not become evident until the summer of 1924. Early the following spring applications of sulfur at the rate of 400 lb. per acre were made to a rather large number of plots, including some of sweet clover, alsike, medium red and mammoth clover as well as to many of alfalfa. In all cases the sulfur application caused a darker color, taller growth and heavier yield. After the first cutting of hay in 1925, one-half of each of 95 plots sown in 1922 that had previously received no sulfur or gypsum was treated with gypsum at the rate of 200 lb. per acre. The effect in every case was distinct. During the following winter and spring the mortality of the plants was so high that most of the plots were plowed and reseeded. On 16 plots left the yields were very light, less than a ton to the acre from two cuttings, this being due to the combined effects of the winter injury and an unusually severe and prolonged summer drouth. The gypsum treated plots showed no distinct superiority in resistance to the winter injury or in yield following this.

TABLE 8.—*Effect of sulfur fertilizers on alfalfa, at Bemidji. Fertilizers applied last week in October 1924 or first week in April 1925*

Application with rate per acre	No. of plots	1925		1926		Total for 2 years (tons)
		First cutting (tons)	Second cutting (tons)	First cutting (tons)	Second cutting (tons)	
1. North field. Alfalfa seeded in 1923						
None	6	1.33	0.40	0.62	0.40	2.75
Magnesium sulfate, 380 lb.	3	1.82	.59	.88	.58	3.87
Increase		.49	.19	.26	.18	1.12
Sodium sulfate, 500 lb.	3	2.07	.63	.93	.55	4.18
Increase		.74	.23	.31	.15	1.43
Gypsum, 220 lb.	3	2.05	.60	1.07	.58	4.30
Increase		.72	.20	.45	.18	1.55
Gypsum, 1000 lb.	3	1.90	.66	1.06	.64	4.26
Increase		.57	.26	.44	.24	1.51
Sulfur, 50 lb.	3	1.96	.60	.96	.55	4.07
Increase		.63	.20	.34	.15	1.32
Sulfur, 100 lb.	3	1.97	.64	1.01	.61	4.23
Increase		.64	.24	.39	.21	1.48
2. Main field. Alfalfa seeded in 1922						
Marl, 4 tons	3	1.21	0.44	.50	.37	2.52
Marl, 2 tons; gypsum, 500 lb.	3	2.08	.80	1.40	.78	5.06
Increase		.87	.36	.90	.41	2.54
Limestone, 3 tons	3	1.30	.57	.71	.50	3.08
Limestone, 2 tons; gypsum, 500 lb.	3	2.25	.83	1.42	.86	5.36
Increase		.95	.26	.71	.36	2.28

## SULFUR CONTENT OF ALFALFA

The sulfur content was determined in many samples from the second cutting of alfalfa of the 1924 crop and in a few from the first. These had been collected at the time the alfalfa was ready to be mown, dried without exposure to rain or dew and protected from the loss of any leaves and fine stems. In most cases each sample consisted of the entire growth from 6 square yards well distributed over the plot, and freed of any other plants if such were present in the original. The entire sample was dried in the oven, ground in a hammer mill and well mixed before analysis.

The method of analysis used is that adopted by the Association of Official Agricultural Chemists for the determination of sulfur in plants, including the seeds (2, 8). In this the sample is moistened with a solution of magnesium nitrate and heated until oxidation of the organic matter is



complete, after which the sulfur is determined as barium sulfate. This has been found to give as high values as the bomb method. The data reported in Tables 9 and 10 are the averages of concordant duplicate determinations.

TABLE 9.—*Effect of treatment upon sulfur content of alfalfa in 1924*

Application	First plot (percent)	Second plot (percent)	Third plot (percent)	Average (percent)
1. Bemidji, first cutting				
None	0.11	0.10	0.07	0.09
Limestone—3 tons	.11			.11
Do —4 Do	.12	.13		.13
Do —3 Do, phosphate	.13	.11		.12
Do —3 Do, phosphate, potash	.14	.13	.13	.13
2. Bemidji, second cutting				
None	0.10	0.09	0.11 <sup>a</sup>	0.10
Gypsum	.20	.23		.21
Limestone—3 tons	.10	.10	.10 <sup>b</sup>	.10
Do —4 Do	.11	.10		.10
Do —8 Do	.13	.14		.13
Do —3 Do, manure 10 tons	.17	.15		.16
Do —3 Do, gypsum	.22	.22		.22
Do —3 Do, phosphate, potash	.12	.12	.12	.12
Do —3 Do, phosphate, potash, gypsum	.17	.18		.17
3. Backus, first cutting				
None	0.12	0.12	0.12	0.12
Limestone—3 tons, phosphate, potash	.12	.12	.14	.13
Do —3 Do, phosphate	.13	.16		.14
Do —4 Do	.12	.14	.14	.13
4. Backus, second cutting				
None	0.12	0.14	0.15	0.14
Gypsum	.24	.30		.27
Limestone—3 tons	.14	.14	.15	.14
Do —3 Do, gypsum	.26	.26	.24	.25
Do —3 Do, phosphate, potash	.13	.12	.14	.13
Do —3 Do, phosphate, potash, gypsum	.21	.20	.24	.22
Do —3 Do, phosphate	.16			.16
Do —4 Do	.15			.15
Do —8 Do	.17	.16		.16

<sup>a</sup> The sample from a fourth plot contained 0.10 per cent S.

<sup>b</sup> Do 0.11 Do

TABLE 9 (Continued).—Effect of treatment upon sulfur content of alfalfa in 1924

Application	First plot (percent)	Second plot (percent)	Third plot (percent)	Average (percent)
-------------	----------------------------	-----------------------------	----------------------------	----------------------

## 5. Coon Creek, second cutting

None	0.32	0.34	0.35	0.34
Gypsum	.40	.38	.37	.38
Limestone—3 tons	.32	.32	.29	.31
Do —3 Do, gypsum	.41	.35	.41	.39
Do —3 Do, phosphate, potash	.32	.33	.30	.32
Do —3 Do, phosphate, potash, gypsum	.38	.38	.34	.37

## 6. Voxland, second cutting

None	0.33	0.31	0.32	0.32
Gypsum	.38	.39		.38
Limestone—4 tons	.36	.36		.36
Do —4 Do, gypsum	.38	.36	.41	.38
Do —4 Do, phosphate, potash	.36	.35	.32	.34
Do —4 Do, phosphate, potash, gypsum	.35	.36	.36	.36
Do —8 Do	.34	.36	.36	.35

## 7. Foss, second cutting

None	0.32	0.38	0.33	0.34
Gypsum	.41	.36	.37	.38
Limestone—4 tons	.37	.36		.36
Do —4 Do, gypsum	.42	.38	.36	.38
Do —4 Do, phosphate, potash	.31	.32		.31
Do —4 Do, phosphate, potash, gypsum	.34	.30	.32	.32
Do —8 Do	.37	.37	.37	.37

## 8. Caledonia, second cutting

None	0.26	0.32		0.29
Limestone—4 tons	.24	.22	.22	.23
Do —4 Do, gypsum	.23	.23	.24	.23
Do —4 Do, phosphate, potash	.25	.24		.24
Do —4 Do, phosphate, potash	.23	.22	.19	.21

## 9. Hayfield, second cutting

None	0.50	0.43	0.46	0.46
Sulfur	.45	.50		.47
Sulfur and rock phosphate	.48	.54		.52

TABLE 10.—*Sulfur content of second cutting of alfalfa hay at various experimental fields in 1924. Summary of averages*

Application	Bemidji	Backus	Coon Creek	Voxland	Foss	Caledonia	Hayfield
	per cent	per cent	per cent	per cent	per cent	per cent	per cent
None	0.10	0.14	0.34	0.32	0.34	0.29	0.46
Limestone—3 tons	.10	.16	.31				
Do 4 Do	.10	.15		.36	.36	.23	
Do 8 Do	.13	.16		.35	.37		
Limestone, phosphate, potash	.12	.13	.32	.34	.31	.24	
Limestone, gypsum	.22	.25	.39	.38	.36	.23	
Gypsum	.21	.27	.38	.38	.37		
Limestone, phosphate, potash, gypsum	.17	.22	.37	.36	.32	.21	
Sulfur							.47
Sulfur and rock phosphate							.52
Lowest average	.10	.13	.31	.32	.31	.21	.52
Highest average	.22	.27	.39	.38	.37	.29	.46
Lowest individual sample	.09	.12	.29	.31	.30	.19	.43
Highest individual sample	.23	.30	.40	.41	.42	.29	.54

## THE CONTROL PLOTS IN 1924

In the case of the untreated plots there was a very wide variation from field to field (Table 11), but with the samples from plots on the same field it was narrow and most so with those on which the sulfur content was lowest. At Bemidji it was lowest of all, at Backus a little higher, at Hayfield over four times as high and at the four other fields about three times as high. The difference is not connected with differences in the texture of the soils, as at Coon Creek, with the coarsest soil of all, the content was as high as on three of the fields with fine textured soils (Table

TABLE 11.—*Sulfur content of alfalfa from untreated plots in 1924 with data arranged to show the minima and maxima*

Plot	Field and Cutting								
	Bemidji		Backus		Coon Creek	Voxland	Foss	Caledonia	Hayfield
	1st	2nd	1st	2nd	2nd	2nd	2nd	2nd	2nd
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
Lowest	0.07	0.09	0.12	0.12	0.32	0.31	0.32	0.26	0.43
Intermediate	.10	.10	.12	.14	.34	.32	.33		.46
Highest	.11	.11	.12	.15	.35	.33	.38	.32	.50
Average	.09	.10	.12	.14	.34	.32	.34	.29	.46

5). Neither is it due to there being a heavier crop on the first two fields with a resultant heavier demand on the sulfur, as may be seen from Table 12. The sulfur content in the hay from the treated plots was very low on the two fields that have shown a response to sulfur fertilizers and high on the others. Comparing the two that show a response it will be seen that the sulfur content is the lower at Bemidji, the field on which there is the greatest response.

TABLE 12.—Yield of hay of second crop of alfalfa on control plots in 1924 and amount of sulfur per acre removed by crop

Field	Yield per acre	Sulfur content	Sulfur removed
	tons	per cent	per acre lb.
Bemidji	0.44	0.10	0.9
Backus	.79	.14	2.2
Coon Creek	.70	.34	4.8
Voxland	1.83	.32	11.7
Foss	1.43	.34	9.7
Caledonia	1.16	.29	6.7
Hayfield	.69	.46	6.3

### EFFECT OF GYPSUM IN 1924

The effect of the very heavy application of gypsum is shown in Table 13. In this there are reported, in the case of Caledonia, data from the

TABLE 13.—Sulfur content of alfalfa from plots given 1000 lb. per acre of gypsum. Second cutting in 1924

1. Sulfur content of crop							
	Bemidji	Backus	Coon Creek	Voxland	Foss	Caledonia*	Hay-field
	per cent	per cent	per cent	per cent	per cent	per cent	per cent
Lowest	0.20	0.24	0.37	0.38	0.36	0.23	0.45
Intermediate			.38		.37	.23	.48
Highest	.23	.30	.40	.39	.41	.24	.54
Average	.21	.27	.38	.38	.38	.23	.49
2. Increase in sulfur content due to fertilization							
Average	0.11	0.13	0.04	0.06	0.04	-0.06	0.03
3. Relative sulfur content of fertilized alfalfa							
Average	210	193	112	119	118		107

\* Given 200 lb. of sulfur instead of 1000 lb. of gypsum.

plots given both gypsum and limestone, because there were none with gypsum only, and in the case of Hayfield from the sulfur treated plots because no analyses were made of samples from those treated with gypsum. All are from the second cutting. At Bemidji and Backus the sulfur content was about doubled while at the others it was raised less than one-fifth. At Caledonia, where it was even lower on the treated than on the untreated plots, the difference may be considered within the range of experimental error. On none of the fields, except those at Bemidji and Backus, would the analyses serve to suggest which samples were from treated plots.

The effect of gypsum was much the same when applied along with limestone, or with limestone, phosphate and potash, increasing the sulfur content of the crop to about the same extent, except where the other applications caused a distinct increase in yield, in which case the sulfur content was lower than where the gypsum was used alone.

#### EFFECT OF LIMESTONE

At Caledonia and Coon Creek, at both of which fields the application of limestone alone increases the yield of alfalfa, it slightly decreased the sulfur content. On the Voxland and Foss fields it slightly increased it. At Bemidji and Backus, where limestone distinctly increases the yields, it in general increased the sulfur content of the alfalfa, and so caused a decided increase in the amount of sulfur removed in the crop.

It is important to point out that the limestone used in these experiments contained an appreciable amount of sulfur. No sample was saved from the lot used in 1921 but a sample of the product of the same limestone plant secured in 1924 carried 0.20 per cent, which is equivalent to 21.5 lb. of gypsum per ton. As from 3 to 8 tons per acre of limestone was applied the incidental application of sulfur was probably equivalent to from 64 to 172 lb. of gypsum per acre, which although small compared with the 1000 lb. application is of importance when used on such a sulfur-hungry soil as that of the Bemidji field. The influence of the sulfur thus supplied upon the yields of the Bemidji plots is discussed in a later section of the paper.

#### EFFECT OF PHOSPHATE, POTASH AND MANURE

The sulfur content was influenced by phosphate and potash applications but slightly and then in general indirectly, an increase in yield being accompanied by a slight decrease in the sulfur content. Manure at Bemidji distinctly raised the sulfur content while also increasing the yields.

#### ALFALFA AT BEMIDJI IN 1925

At Bemidji in 1925 the weather was very favorable for the first crop of alfalfa, 12.3 inches of rain falling between April 1 and the end of June,

when the first crop was cut, but dry weather following this caused a light second crop. Sulfur determinations were made on many samples of both cuttings, chiefly from the North Field, which had been under cultivation about 10 years, only about half as long as the Main Field, and which had not been seeded until a year later. This field had been treated with marl at the rate of 3 tons per acre, but not laid out in plots or given any sulfur fertilizer until the end of October, 1924.

#### SULFUR CONTENT OF FIRST CUTTING

Among the samples taken from the first cutting on the Main Field were those from the same three control plots and the two plots treated with gypsum reported in Part 2 of Table 9. The sulfur content (Table 14) was about half as high again on both as it had been on the second cutting in 1924, while the yield on the control plots was about two and a half times as high, making the removal of the sulfur per unit area about four times as high.

TABLE 14.—*Sulfur content and yield of first cutting of alfalfa in 1925 on Main Field at Bemidji*

	First plot	Second plot	Third plot	Average
Sulfur content				
On controls, per cent	0.16	0.16	0.14	0.15
With gypsum, per cent	.32	.32	.28	.31
Yield per acre of hay				
On controls, tons	1.33	1.15	1.15	1.21
With gypsum, tons	1.72	1.98	1.94	1.88
Removal of sulfur per acre				
On controls, lb.	4.3	3.7	3.2	3.7
With gypsum, lb.	11.0	12.7	10.9	11.5

The sulfur content on the control plots, while much higher than in either cutting in 1924, was lower than found in the second cutting in 1924 on the control plots on the four fields that showed no increase in yield from gypsum applications.

At the end of October, 1924, magnesium sulfate at the rate of 400 lb. per acre, equivalent to 65 lb. of sulfur, was applied on each of 8 plots on the Main Field that had been variously treated with ground limestone in 1921 and sown to alfalfa in the summer of 1924. Samples from the first cutting were analyzed and found to contain from 0.27 to 0.30 per cent sulfur (Table 15). The application of limestone appeared to exert no influence on the sulfur content of the alfalfa.

In Table 16 are reported the analyses of 21 samples from the same cutting on the North Field. Eight of these are from controls and are similar in sulfur content to the samples of the same cutting from the

TABLE 15.—Sulfur content of alfalfa treated with 400 lb. per acre of magnesium sulfate. First cutting in 1925

Application of limestone, per acre	Sulfur content		
	First plot	Second plot	Average
None	per cent 0.30	per cent 0.28	per cent 0.29
2 tons	.32	.30	.31
3 tons	.27	.26	.26
4 tons	.30	.30	.30

controls on the Main Field (Table 14), averaging 0.16 per cent. Salt did not affect it but sulfur flour, gypsum and sodium sulfate all increased the sulfur content, generally about 100 per cent. It shows little dependence upon the rate of application, which in all cases was heavy.

TABLE 16.—Effect of sulfur fertilizers upon sulfur content and yield of first cutting of alfalfa in 1925 on North Field at Bemidji

Plot	Application with rate per acre		Sulfur content of first cutting	Yields per acre	
				First cutting	Second cutting
		lb.	per cent	tons	tons
1	None		0.16	1.26	0.53
2	Do		.15	1.15	.40
3	Do		.16	1.63	.41
4	Do		.15	1.12	.37
5	Do		.19	1.64	.45
6	Do		.18	1.39	.43
7	Do		.15	1.35	.46
8	Do		.15	1.47	.48
Average			.16	1.38	.44
9	Sodium chloride	360	.15	1.53	.42
10	Gypsum	1000	.36	2.04	.68
11	Do	1000	.38	1.98	.67
12	Do	1000	.38	2.54	.63
13	Do	1000	.32	2.31	.61
14	Do	1000	.38	2.26	.57
Average			.36	2.25	.63
15	Sodium sulfate	500	.28	2.03	.56
16	Do	1000	.29	2.02	.61
17	Sulfur flour	100	.34	1.75	.55
18	Do	200	.35	2.09	.63
19	Do	400	.43	1.96	.74
20	Do	400	.40	1.89	.75
21	Do	400	.38	2.03	.52
Average			.40	1.96	.67

## SECOND CUTTING IN 1925 ON NORTH FIELD

The second cutting was removed during the first week in September. A considerable number of plots on the North Field were treated with

TABLE 17.—Effect of sulfur fertilizers upon sulfur content and yield of second cutting of alfalfa on North Field at Bemidji in 1925. First cutting, June 26 to July 1. Applications of fertilizers made July 17 to 21. Second cutting, September 4 to 9. No fertilization on any plot until after first cutting

Plot	Application	Rate per acre	Equivalent amount of sulfur	Sulfur content	Yield per acre	
					First cutting	Second cutting
		lb.	lb.	per cent	tons	tons
1	None	0	0	0.21	0.99	0.40
2	Do	0	0	.23	1.04	.50
3	Do	0	0	.19	1.15	.41
4	Do	0	0	.22	1.12	.41
5	Do	0	0	.18	1.29	.44
6	Do	0	0	.19	1.46	.45
7	Do	0	0	.20	1.34	.48
8	Do	0	0	.20	1.56	.38
9	Do	0	0	.20	1.12	.37
10	Do	0	0	.18	"	.50
11	Do	0	0	.18	"	.46
12	Do	0	0	.19	"	.47
13	Do	0	0	.20	"	.36
14	Do	0	0	.21	"	.40
15	Do	0	0	.18	"	.51
Average		0	0	.20	1.27	.44
16	Gypsum	50	9.3	.30	0.86	.66
17	Do	50	9.3	.30	"	.56
18	Do	50	9.3	.31	"	.65
Average				.30		.62
19	Gypsum	100	18.6	.34	0.86	.65
20	Do	100	18.6	.38	"	.64
21	Do	100	18.6	.37	"	.65
Average				.36		.65
22	Gypsum	200	37.2	.48	0.86	.76
23	Do	200	37.2	.41	"	.71
24	Do	200	37.2	.40	"	.66
Average				.43		.71
25	Gypsum	500	93.0	.40	"	.71
26	Do	1080	200.9	.76	"	.79
27	Sulfur flour	25	25.0	.30	"	.80
28	Do	25	25.0	.30	"	.87
29	Do	25	25.0	.28	"	.78
Average				.29		.82

\* Plot was part of undivided field and not harvested separately at time of first cutting.



**TABLE 17 (Continued).—Effect of sulfur fertilizers upon sulfur content and yield of second cutting of alfalfa on North Field at Bemidji in 1925. First cutting June 26 to July 1. Applications of fertilizers made July 17 to 21. Second cutting, September 4 to 9. No fertilization on any plot until after first cutting**

Plot	Application	Rate per acre	Equivalent amount of sulfur	Sulfur content	Yield per acre	
					First cutting	Second cutting
		lb.	lb.	per cent	tons	tons
30	Sulfur flour	50	50	0.42	"	0.67
31	Do	50	50	.37	"	.87
32	Do	50	50	.46	"	.85
33	Do	50	50	.36	"	.70
Average				.40		.80
34	Sulfur flour	75	75	.46	"	.68
35	Do	100	100	.44	"	.65
36	Do	200	100	.44	"	.93
37	Do	100	100	.44	"	.94
38	Do	100	100	.41	"	.74
Average				.43		.81
39	Sulfur flour	200	200	.44	"	.87

\* Plot was part of undivided field and not harvested separately at time of first cutting.

sulfur flour and gypsum about the middle of July, 3 weeks after the first crop had been mown, and analyses made of samples from 24 of these and also of samples from 15 of the control plots (Table 17). On account of the

**TABLE 18.—Effect of sulfur fertilizers upon sulfur content of first cutting of alfalfa in 1925 on Backus and Caledonia fields**

Field	Application with rate per acre	Sulfur content			
		First plot	Second plot	Third plot	Average
		per cent	per cent	per cent	per cent
Backus	Marl, 4 tons	0.16	0.18	0.18	0.17
Do	Limestone, 3 tons	.19	.15	.19	.18
Do	Do, 3 tons; gypsum, <sup>a</sup> 1000 lb.	.28	.28	.28	.28
Do	Marl, 4 tons; sulfur, <sup>b</sup> 400 lb.	.40	.41	.44	.42
Caledonia	Limestone, 4 tons	.29	.27		.28
Do	Do, 4 tons; gypsum, <sup>a</sup> 1000 lb.	.31	.32	.30	.31
Do	Do, 4 tons; phosphate, pot-ash, gypsum, <sup>c</sup> 1000 lb.	.30	.28	.32	.30

<sup>a</sup> Applied in 1921.

<sup>b</sup> Applied in April, 1925.

<sup>c</sup> Applied in 1922.

late date of the application the fertilizers did not have an opportunity to exert their full effect.

The yield, which was light, averaging only 0.44 ton on the control plots, was increased by 50 to 100 per cent by the applications. Twenty-five pounds per acre of sulfur flour had as marked an effect as any heavier application upon the yield but did not raise the sulfur content as much. Fifty pounds per acre of gypsum increased both yield and sulfur content about 50 per cent. With the heavier applications the sulfur content was doubled.

In the case of the samples from Backus the sulfur content was a little higher than in the second cutting of the year before, as was also the case with samples from the Caledonia field (Table 18). At Backus the gypsum application continued to greatly affect the sulfur content while at Caledonia it again showed only a very slight influence.

#### DISTRIBUTION OF SULFUR BETWEEN LEAVES AND STEMS OF ALFALFA

The occasions when analyses of alfalfa may prove most valuable in throwing light upon the probable need of sulfur fertilization often arise when it is not possible to secure samples of entire plants that have been protected from all loss of leaves and fine stems. For this reason, and also because of the possibility that the sulfur content of either the stems alone or that of the leaves might prove a better indicator of sulfur deficiency than that of the entire plant, leaves and stems were separated from a large number of samples and the sulfur in both determined. The samples were collected chiefly from the first cutting in 1925 at Bemidji, Backus and Caledonia. The plants used as a sample from a plot were gathered from a representative square yard and dried without loss of leaves. Then the leaves and very fine stems were stripped off, taking the stems one by one in the one hand and drawing them through the fingers of the other. With each sample the percentage of leaves, including the very fine stems, was determined and is reported in Table 19.

The leaves constitute about half the entire weight, the plants varying in maturity from one-quarter to full bloom. The sulfur fertilizer shows no distinct influence upon the proportion of leaves except with the 6 plots of the 1922 seeding on the Main Field at Bemidji. This exception may have no importance but it should be pointed out that it occurs on the oldest seeding on the most sulfur deficient field dealt with in this paper.

In 1925 wherever the sulfur fertilizers caused an increased growth they increased the height of the alfalfa, delayed its maturity and caused a dark green color. As the plants were less mature they contained more water and the differences in yields between fertilized and untreated plots were much greater when the comparisons were based upon the weights of

the fresh samples. This tended to exaggerate the apparent benefit of the fertilization.

In each of the 67 samples analyzed the sulfur content of the leaves was much higher than that of the stems. On the plots not treated with the sulfur fertilizer it was from 1.8 to 2.3 times as high, being the same on the very sulfur hungry Main Field at Bemidji as on the unresponsive field at Caledonia. On the latter the ratio was not affected by sulfur fertilization

TABLE 19.—Influence of fertilization upon distribution of sulfur between leaves and stems of alfalfa. Crops of 1925

Application with rate per acre	Plot	Proportion of entire weight in form of leaves	Date of cutting (per cent)	Maturity * (per cent)	Sulfur content			Ratio of S in leaves S in stems
					Whole plant (per cent)	Leaves (per cent)	Stems (per cent)	
1. Bemidji, seeding of 1922 on Main Field, first cutting								
None	1	54.5	June 26	75	0.16	0.21	0.09	2.4
Do	2	53.6	Do 27	75	.16	.22	.10	2.1
Do	3	54.2	Do 29	75	.18	.19	.09	2.0
Average		54.1		75	.17	.20	.09	2.2
Gypsum, 1000 lb.	1	47.7	Do 26	25	.32	.48	.18	2.7
	2	45.5	Do 27	25	.32	.49	.17	2.8
	3	46.0	Do 29	50	.28	.42	.16	2.6
Average		46.4		33	.31	.46	.17	2.7
2. Bemidji, seeding of 1923 on North Field, first cutting								
None	1	46.5	June 30	100	0.16	0.21	0.12	1.8
Do	2	45.5	July 1	100	.15	.21	.11	1.9
Do	3	44.7	Do 1	100	.16	.24	.10	2.4
Do	4	41.6	Do 1	100	.15	.21	.11	1.9
Do	5	50.8	June 30	100	.19	.26	.12	2.1
Do	6	45.5	July 1	100	.18	.24	.13	1.9
Do	7	45.8	Do 1	100	.15	.21	.11	2.0
Do	8	42.9	Do 1	100	.15	.20	.11	1.9
Average		45.4		100	.16	.22	.11	2.0
Sodium chloride, 360 lb.	1	42.2	July 1	100	.16	.24	.10	2.5
Gypsum, 1000 lb.	1	49.3	June 30	50	.36	.53	.18	2.8
Do	2	41.5	July 1	50	.38	.61	.21	2.8
Do	3	42.9	Do 1	50	.38	.62	.21	3.0
Do	4	43.4	Do 1	50	.32	.60	.22	3.3
Do	5	41.4	Do 1	50	.38	.56	.25	2.2
Average		43.7		50	.36	.58	.21	2.8
Sulfur flour, 100 lb.	1	45.8	Do 1	50	.34	.50	.20	2.5
Do 200 Do	1	43.1	Do 1	50	.35	.58	.19	3.0
Do 400 Do	1	46.5	June 30	50	.43	.65	.25	2.6
Do 400 Do	2	48.0	July 1	75	.40	.61	.22	2.8
Do 400 Do	3	45.0	Do 1	50	.38	.60	.20	3.0
Average		46.5		58	.40	.62	.22	2.8
Sodium sulfate, 500 lb.	1	40.5	July 1	50	.28	.45	.16	2.7
Do 1000 Do	1	41.0	Do 1	50	.29	.48	.16	3.0

\* Estimated proportion of plants in bloom.

TABLE 19 (Continued).—Influence of fertilization upon distribution of sulfur between leaves and stems of alfalfa. Crops of 1925

Treatment	Plot	Proportion of entire weight in form of leaves	Date of cutting (per cent)	Maturity (per cent)	Sulfur content			Ratio of S in leaves S in stems
					Whole plant (per cent)	Leaves (per cent)	Stems (per cent)	
3. Bemidji, seeding of 1923 on North Field, second cutting								
None	1	55.7	Sept. 5		0.18	0.22	0.12	1.8
Do	2	56.5	Do		.18	.23	.12	1.8
Average		56.1			.18	.22	.12	1.8
Gypsum, 50 lb.	1	58.2	Sept. 5		.29	.37	.17	2.2
Do 100 lb.	1	56.0	Do		.33	.44	.19	2.3
Do 200 lb.	1	53.8	Do		.34	.46	.21	2.2
Sulfur, 25 lb.	1	59.7	Sept. 5		.28	.36	.17	2.1
Do 50 lb.	1	61.0	Do		.36	.44	.21	2.1
Do 100 lb.	1	59.0	Do		.41	.52	.25	2.1
Do 200 lb.	1	60.7	Do		.44	.58	.24	2.4
4. Bemidji, seeding of 1924 on Main Field, first cutting								
Magnesium sulfate, 400 lb.	1	51.1	June 29	50	0.30	0.43	0.16	2.7
Do	2	50.3	Do	50	.28	.40	.15	2.7
Average		50.7		50	.29	.41	.15	2.7
Limestone, 2 tons; magne- sium sulfate, 400 lb.	1	52.5	June 29	50	.32	.46	.17	2.7
Do	2	51.5	Do	50	.30	.44	.16	2.8
Average		52.0		50	.31	.45	.16	2.7
Limestone, 3 tons; magne- sium sulfate, 400 lb.	1	54.8	June 29	50	.27	.39	.15	2.5
Do	2	56.3	Do	50	.26	.37	.14	2.7
Average		55.5		50	.26	.38	.14	2.6
Limestone, 4 tons; magne- sium sulfate, 400 lb.	1	53.2	June 29	50	.30	.40	.17	2.4
Do	2	51.7	Do	50	.30	.44	.16	2.8
Average		52.4		50	.30	.42	.16	2.6
5. Bemidji, seeding of 1925 on South Field,* first cutting								
Phosphate and potash	1	60.6	Sept. 15		0.26	0.33	0.14	2.3
Do	2	61.8	Do		.23	.30	.13	2.3
Do	3	60.6	Do		.23	.34	.15	2.3
Average		61.0			.24	.32	.14	2.3
Phosphate, potash and gyp- sum, 1000 lb.	1	59.4	Sept. 15		.33	.42	.20	2.3

\* Alfalfa on the South Field which has been under cultivation a shorter time and has been more heavily manured, has not shown sulfur hunger in the 2 years it has been under experiment.

while on the responsive Bemidji and Backus fields it was distinctly raised, in many cases to as much as 2.7. On the South Field at Bemidji, which has been under cultivation a much shorter time than the Main and North Fields and has also been more frequently manured, alfalfa was seeded in

TABLE 19 (Continued).—Influence of fertilization upon distribution of sulfur between leaves and stems of alfalfa. Crops of 1925

Treatment	Plot	Proportion of entire weight in form of leaves	Date of cutting (per cent)	Maturity (per cent)	Sulfur content			Ratio of S in leaves S in stems
					Whole plant (per cent)	Leaves (per cent)	Stems (per cent)	
6. Backus, seeding of 1922, first cutting								
Limestone, 3 tons	1	46.8	July 6	100	0.19	0.24	0.11	2.2
Do	2	43.4	Do	100	.15	.22	.10	2.1
Do	3	48.9	Do 9	100	.19	.24	.14	1.8
Average		46.4		100	.18	.23	.12	2.0
Limestone, 3 tons and gyp- sum, 1000 lb.	1	47.1	July 7	90	.28	.43	.15	2.8
Do	2	46.6	Do 9	90	.28	.42	.17	2.5
Do	3	47.0	Do 6	90	.28	.44	.17	2.7
Average		46.9		90	.28	.43	.16	2.7
7. Backus, seeding of 1924, first cutting								
Marl, 4 tons	1	45.1	July 6	100	0.16	0.22	0.12	1.8
Do	2	46.6	Do 7	100	.18	.26	.11	2.2
Do	3	48.3	Do 9	100	.18	.25	.17	1.9
Average		46.7		100	.17	.24	.12	2.0
Marl, 3 tons; sulfur, 400 lb.	1	47.1	July 6	90	.40	.63	.20	3.1
Do	2	50.9	Do 6	90	.41	.57	.22	2.7
Do	3	46.0	Do 9	90	.44	.67	.25	2.7
Average		48.0		90	.42	.63	.22	2.8
8. Caledonia, seeding of 1922, first cutting								
Limestone, 4 tons	1	55.2	July 1	100	0.29	0.39	0.17	2.3
Do	2	52.6	Do	100	.27	.37	.16	2.2
Average		53.9		100	.28	.38	.16	2.2
Limestone, 4 tons; gypsum 1000 lb.	1	52.7	July 1	100	.31	.41	.20	2.1
Do	2	54.0	Do	100	.32	.43	.18	2.3
Do	3	58.1	Do	100	.30	.40	.18	2.2
Average		54.9		100	.31	.41	.18	2.2
Limestone, 4 tons; phosphate, potash, gypsum, 1000 lb.	1	52.7	July 1	100	.30	.41	.18	2.1
Do	2	56.5	Do	100	.28	.38	.16	2.3
Do	3	54.1	Do	100	.32	.43	.18	2.3
Average		54.4		100	.30	.40	.18	2.2

April, 1925, part of the plots receiving 1000 lb. per acre of gypsum. In the three cuttings since made the gypsum has shown no benefit and with the cutting made in September of the year of seeding the ratio of the sulfur in the leaves to that in the stems was found to have not been affected (Part 5, Table 19).

Whenever shattering has not taken place it appears better to compare the sulfur content of the leaves of the alfalfa rather than that of the entire plants or of the stems because their sulfur content is highest and also most affected by the sulfur supply of the soil. Wherever any serious loss of

leaves has occurred the comparison of analyses of entire samples appears inadmissible and it then is necessary to separate the remaining leaves from the stems and analyze the one or both, but not the mixture. If practically no leaves remain the analysis of the stems fully stripped may give useful data.

The use of the entire plant, where no shattering has occurred, has the advantage that it lessens the labor of the preparation of the samples and permits a comparison with data from previous analyses in the same laboratory and with those reported from elsewhere.

### EFFECT OF SULFUR UPON THE COLOR AND EARLINESS OF MATURITY OF ALFALFA

It has already been mentioned that the general effect of sulfur fertilizers upon alfalfa on sulfur-deficient soil is to cause a darker color, taller growth and to delay maturity. Neller has observed the same in the state of Washington (13, p. 15). While this appears to be generally true there are important exceptions. On the occasion of three cuttings on the Main Field at Bemidji careful notations were made on the color, average height and estimated proportion of plants in bloom in the case of 45 plots, 16 of which had received neither limestone nor sulfur fertilizer. Twelve had been given gypsum at 1000 lb. per acre in 1921 and the rest had received varying amounts of limestone alone or in combination with potash or phosphate or with both. With all three cuttings the gypsum caused a dark green color, increased the average height of the plants and markedly increased the yield. On the first cutting in 1925 and the second in 1926 it delayed maturity but with the first in 1926 it distinctly hastened it. As there were triplicate plots with each application and four sets of triplicate controls the averages of triplicates are reported in Table 20.

TABLE 20.—*Effect of sulfur fertilizers upon maturity and height of plants on Main Field at Bemidji. A, June 26, 1925. B, June 29, 1926. C, August 5, 1926*

Application with rate per acre	No. of plots	Yield of crop			Average height of plants			Proportion of plants in bloom		
		A	B	C	A	B	C	A	B	C
		tons	tons	tons	in.	in.	in.	per cent	per cent	per cent
None	3	1.10	0.49	0.34	15	10	11	66	28	83
Do	3	1.21	0.48	0.36	19	11	11	75	33	100
Do	3	1.47	0.62	0.45	21	11	13	66	33	83
Do	3	1.43	0.59	0.39	20	9	11	75	25	75
Marl, 4 tons	3	1.21	0.51	0.37	19	11	14	75	28	100
Gypsum, 1000 lb. in 1921	3	1.88	1.35	0.84	24	20	22	33	50	50
Do 500 lb. in April, 1925	3	2.21	1.39	0.79	24	22	20	25	50	50
Limestone, 3 tons	3	1.30	0.71	0.49	20	12	15	66	50	100
Do 3 tons; gypsum 1000 lb.	3	2.05	1.66	0.89	24	21	21	28	50	50
Limestone, phosphate and potash	3	1.56	1.03	0.57	21	19	19	66	58	100
Do Do potash and gypsum	3	2.37	1.72	0.99	24	23	23	25	58	42
Limestone, 8 tons	3	1.78	1.03	0.56	23	16	19	50	50	100

## SULFUR CONTENT OF CLOVERS

Gypsum has been tried with the common clovers and sweet clover on the Main Field at Bemidji and on the Backus Field. It has shown much the same effect as with the alfalfa on the same fields, viz.: taller growth, darker green color, heavier yield and higher sulfur content, but the frequent serious losses so common with clovers from winter killing seriously interfere with the use of these in such studies. Sweet clover in the early part of its second year shows about as striking a response as alfalfa but the opportunity for a satisfactory comparison in yields on any two plots sown to this as a trial crop would usually be limited to the first cutting of the second year. In the case of an established field of alfalfa during

TABLE 21.—Effect of sulfur fertilizers upon yield and sulfur content of clovers compared with alfalfa

1. Yields on Main Field at Bemidji						
Crop	1925			1926		
	Yield per acre		Relative yield on fertilized plots <sup>a</sup>	Yield per acre		Relative yield on fertilized plots
	Unfertilized plots	Fertilized plots		Unfertilized plots	Fertilized plots	
Alfalfa, sown in 1923	tons 2.45	tons 3.36	136	tons 1.19	tons 2.08	175
Sweet clover	.91	1.33	146	1.44	1.63	113
Medium red clover	.75	2.54	339	.80	1.29	151
Mammoth clover				.89	1.38	155
Alsike clover	.95	2.21	233	.83	1.00	120

2. Sulfur content of first cutting in 1925						
	At Bemidji			At Backus		
	Unfertilized	Fertilized <sup>b</sup>	Ratio of S content	Unfertilized	Fertilized <sup>c</sup>	Ratio of S content
	per cent	per cent		per cent	per cent	
Alfalfa, sown in 1922	0.16	0.36	2.2	0.17	0.42	2.4
Sweet clover	.14	.44	3.0			
Medium red clover	.12	.23	1.9	.15	.24	1.6
Mammoth clover				.15	.25	1.7
Alsike clover	.15	.30	2.0	.15	.26	1.7

<sup>a</sup> Yield on unfertilized plots=100.

<sup>b</sup> 500 lb. per acre of gypsum in April, 1925.

<sup>c</sup> 400 lb. per acre of sulfur flour in April, 1925.

this 2 year period there would be from 4 to 6 crops upon which the effect, if any, would be shown. Table 21 serves to show the effect of sulfur fertilization upon the clovers sown right beside alfalfa similarly treated.

### LIMESTONE A DILUTE SULFUR FERTILIZER

Compared with 4 tons per acre of marl the applications of 3 tons per acre of limestone slightly increased the yield (Table 22) and the average height of the plants, caused a slightly darker color and sometimes slightly hastened maturity. Samples of ground limestone from the same crushing plant were later found to carry 0.20 per cent of sulfur. The marl used contained only 0.0125 per cent. So we may assume that the 3 tons of limestone furnished 12 lb. per acre of sulfur and the 4 tons of marl only 1 lb. The alfalfa on the 3 plots given 8 tons per acre of limestone in 1921 has yielded more heavily than that on those receiving 3 or 4 tons and also more than those given 4 or 8 tons of marl, although the latter carried a higher content of carbonate than the limestone. On the marled plots the plants were similar in color, height and maturity to those on the untreated plots, while with the 8 tons of limestone they approached in appearance those given the gypsum. As the 8 tons of limestone would carry 32 lb. of sulfur, equivalent to 172 lb. of gypsum, the decided superiority of the limestone over the marl is explained. In view of the fact that the 4 and 8 ton applications of marl have had so slight a beneficial effect upon the alfalfa the benefit of the limestone at Bemidji should be attributed to its sulfur content.

TABLE 22.—Comparison of effect of increasing amounts of limestone and marl upon yields of alfalfa at Bemidji

Application	1923	1924		1925		1926		Total for 4 years
	One cutting	First cutting	Second cutting	First cutting	Second cutting	First cutting	Second cutting	
None	tons 0.81	tons 0.70	tons 0.48	tons 1.21	tons 0.46	tons 0.48	tons 0.36	tons 4.50
Marl, 2 tons	.96	.79	.50	"				
Do 4 Do	1.02	.75	.45	1.21	.44	.50	.37	4.74
Do 8 Do	.92	.83	.46	1.13	"			
Limestone, 2 tons	1.10	.80	.61	"				
Do 3 Do	1.00	.75	.61	1.30	.57	.71	.50	5.44
Do 4 Do	1.15	.84	.64	1.47	.62	.79	.50	6.01
Do 8 Do	1.17	1.03	.78	1.77	.70	1.03	.56	7.04

\* Treated with gypsum in April, 1925.

<sup>b</sup> Treated with gypsum in July, 1925

### LITERATURE CITED

- (1) Alway, F. J., Shaw, W. M., and Methley, W. J. 1926. Phosphoric-acid content of crops grown upon peat soils as an index of the fertilization received or required. Jour. Agr. Research [U. S.] 33: 701.



- (2) Association of Official Agricultural Chemists. 1925. Report of sub-committee A on recommendations of referees: sulfur and phosphorus in seeds and plants. *Jour. Assoc. Off. Agr. Chem.* 8: 253.
- (3) Bruce, O. C. 1925. The relation of sulfur to alfalfa production. *Jour. Agr. Research [U. S.]* 30: 937.
- (4) Hall, E. H. 1922. The sulphur and nitrogen content of alfalfa grown under various conditions. *Bot. Gaz.* 73: 401.
- (5) Hart, E. B., and Peterson, W. H. 1911. Sulphur requirement of farm crops in relation to the soil and air supply. *Wis. Agr. Expt. Sta. Res. Bul.* 14.
- (6) ———, and Tottingham, W. E. 1915. Relation of sulphur compounds to plant nutrition. *Jour. Agr. Research [U. S.]* 5: 233.
- (7) Jones, J. S., and Bullis, D. E. 1923. A chemical study of legumes and other forage crops of Western Oregon. *Ore. Agr. Expt. Sta. Bul.* 197.
- (8) Latshaw, W. L. 1923. Report on sulfur and phosphorus in the seeds of plants. *Jour. Assoc. Off. Agr. Chem.* 6: 414.
- (9) Lomanitz, S. 1922. The needs of the soils of Brazos and Jefferson Counties for sulfur. *Tex. Agr. Expt. Sta. Bul.* 302.
- (10) Miller, H. G. 1919. Relation of sulphates to plant growth and composition. *Jour. Agr. Research [U. S.]* 17: 87.
- (11) ———. 1921. Further studies in relation of sulphates to plant growth and composition. *Ibid.* 22: 101.
- (12) Neidig, R. E., McDole, G. R., and Magnuson, H. P. 1923. Effect of sulphur, calcium and phosphorus on the yield and composition of alfalfa on six types of Idaho soils. *Soil Sci.* 16: 127.
- (13) Neller, J. R. 1925. The influence of sulphur and gypsum upon the composition and yield of legumes. *Wash. Agr. Expt. Sta. Bul.* 190.
- (14) Peterson, W. N. 1914. Forms of sulphur in plant materials and their variation with the soil supply. *Jour. Amer. Chem. Soc.* 36: 1290.
- (15) Reimer, F. C., and Tatar, H. V. 1919. Sulphur as a fertilizer for alfalfa in southern Oregon. *Ore. Agr. Expt. Sta. Bul.* 163.
- (16) Shedd, O. M. 1917. Effect of sulphur on different crops and soils. *Jour. Agr. Research [U. S.]* 11: 91.

# NITROGEN ECONOMY IN DUNKIRK SILTY CLAY LOAM

T. L. LYON AND J. A. BIZZELL  
*Cornell University, U. S. A.*

## INTRODUCTION

The studies here reported were made by the use of large lysimeters. Each tank contained about 3.5 tons of soil and presented an area 4 feet, 2 inches square and a depth of 4 feet. The tanks were made of concrete, the surface of which was covered with waterproofing asphalt.

Dunkirk silty clay loam is a glacial lake deposit and agriculturally a soil of medium fertility. The surface soil is somewhat acid but the calcium content becomes greater on descending. The surface soil has only a moderate supply of nitrogen (0.12 per cent). Both surface and subsoil have a tendency to become compact.

When the lysimeter tanks were filled the soil was weighed as it was placed in each tank, and each one-foot layer having been thoroughly mixed, a sample was taken and moisture determined. While this was done for each of the four one-foot layers of soil only the surface foot has been used in making the calculations. At the end of the experiments the soil was removed by layers and was again sampled and analyzed.

Cropping systems with and without legumes and with grass continuously were followed on limed and unlimed soil. Two tanks, one of which was limed, were kept bare of vegetation. The crop rotation without legumes consisted of maize, oats, wheat, and 2 years of timothy hay. The rotation with legumes had red clover seeded with the timothy in the first 5-year period. In the second and third periods soybeans were planted with the maize, field peas with the oats, and red clover with the timothy. Cropping treatments were in duplicate, one of the duplicate tanks being limed and the other remaining untreated with lime. All tanks received the same quantities of farm manure whether cropped or not.

Nitrogen was determined in the manure applied, in the rainfall, in the crops removed, and in the drainage water, also in the soil at the beginning and end of the experiment. The obvious income and outgo are thus accounted for. These data are used in this paper for estimating gains or losses of nitrogen incurred in other ways.

In this presentation of the results of the experiment no general review of related work by other investigators will be made since it is desired to make the paper as brief as possible.

## GAIN OR LOSS OF SOIL NITROGEN NOT ACCOUNTED FOR BY REMOVAL IN CROPS AND DRAINAGE WATER

It has frequently been noted, when total nitrogen has been determined in cultivated soil at the beginning and end of a considerable period of years, that nitrogen has disappeared in greater quantity than can be accounted for by removal in the crops grown. Such a conclusion can be drawn only when the crops are weighed and analyzed throughout a period of considerable length. Determination of the removal of nitrogen by drainage water has not usually been possible in such experiments and hence there has been some uncertainty concerning the relative extent of the removal by drainage and the loss attributable to some less well understood action. In an experiment reported by Russell and Richards (1) analyses of a soil adjoining the Rothamsted drain gauges, and of a later sample taken from the gauges, did not disclose any loss of soil nitrogen that could not be attributed to removal in drainage water. However, field experiments conducted in regions of low rainfall, where little leaching could have taken place, disclosed large losses. For instance, Shutt (2) has shown that of the loss of nitrogen from a virgin prairie soil only one-third could be charged to the crops grown on it. The losses have usually been found in soils high in nitrogen while the soil of the Rothamsted drain gauges is poor in that constituent.

The experiments on the Cornell University lysimeters are well designed to study this subject because the nitrogen removal in the drainage water as well as in the crops has been recorded and the nitrogen added in manure and rainfall is known. For the purpose of measuring the gain or loss of nitrogen not recorded by the data enumerated above four lysimeter tanks were used. Of these, 3 and 7 were planted to a crop rotation consisting of corn, oats, wheat, and then timothy hay for 2 years. They were cropped for 17 years. Tank 3 was not limed. Tank 7 was limed from time to time. The other two tanks, 4 and 8, were kept bare of vegetation for the first 10 years and then cropped to corn, oats, and 3 years of timothy covering in all a period of 15 years. Tank 4 was not limed but Tank 8 received the same quantity of lime as did Tank 7.

In Table 1 nitrogen added to the soil in the form of manure and rainfall is subtracted from the quantity of nitrogen removed in crops and drainage. This gives the gain or loss we might expect to find by soil analysis provided there were no other ways in which nitrogen was removed or added to the soil. From the figures thus obtained were then subtracted the loss as actually found by analysis of the soil at the beginning and at the end of the experiment.

The data show that on cropped soils, on which non-legumes were growing during most of the growing seasons there was no loss of nitrogen that could not be accounted for by the removal in crops and drainage water.

The slight gain shown was well within the limits of experimental error and would not warrant the statement that there was a gain. On the whole, it may be said that there was neither gain nor loss in the soil of these two tanks.

TABLE 1.—*Nitrogen gains or losses in soil bare of plants and in soil planted to non-legumes*  
(Expressed in pounds to the acre)

	Crop rotation of non-legumes, 17 yrs.		No vegetation 10 yrs., cropped 5 yrs.	
	Not limed Tank 3	Limed Tank 7	Not limed Tank 4	Limed Tank 8
Removed in crops and drainage	1129	1075	1001	734
Added in manure and rainfall	939	939	683	683
Difference, or expected loss	190	136	318	51
Actual loss shown by analysis	182	75	689	442
Difference, or unexpected gain or loss	+8	+61	-371	-391
Average annual gain or loss	gain 1/2	gain 3-1/2	loss 25	loss 26

The figures for Tanks 4 and 8 tell a different story. There is a very considerable loss shown by soil analysis that is not accounted for by removal in the crops and drainage water. Since the only respect in which the treatments of Tanks 4 and 8 differed from Tanks 3 and 7 was in being kept free of vegetation during 10 years it would seem that the unexpected loss of nitrogen was probably due to that condition. If that is true, living plants or plant residues must be concerned in some way with the conservation or acquisition of the soil nitrogen not removed in crops or drainage.

### FIXATION OF NITROGEN BY LEGUMES

It is quite evident that the legumes on Tanks 5 and 9 have been instrumental in securing nitrogen from the air. Not only was there more nitrogen in the crops produced but the soil in these tanks contained more nitrogen at the end of the experiment than at the beginning while that in the other tanks contained less.

If from the quantity of nitrogen contained in the crops and drainage water we subtract the nitrogen added in farm manure and rainfall there remains several hundred pounds of nitrogen per acre in excess of that originally in the soil. To this may be added the quantity of nitrogen in the soil at the end of the experiment in excess of that present at the beginning. The figures for this calculation are shown in Table 2.

There were very considerable quantities of nitrogen acquired by the soil during the 15 years of the experiment amounting to 50 lb. as an annual

average for the unlimed soil and 70 lb. for the limed. It probably represents fixation by both legume and by free-living organisms. It will be remembered that after the first 5-year period legumes were planted with each of the cereal crops as well as with timothy. The combination of a legume and non-legume growing at the same time was likely to be favorable for fixation. Absorption of available nitrogen by the non-legume would tend to keep the soil depleted of nitrogen in that form and the legume would probably depend more largely on the nitrogen secured by its symbiotic organisms than if there were more nitrates in the soil. It is well known that a soil poor in nitrogen acquires relatively more nitrogen through a legume crop than does a soil well supplied with nitrogen. The stand of the various legumes was not as dense as if no other plants had been on the soil but there was with each of the last ten crops, except the wheat in 1922, a large proportion of legumes.

TABLE 2.—*Nitrogen gains and losses in soil on which legumes were grown in the rotation (Expressed in pounds to the acre for a period of 15 years)*

	Not limed Tank 5	Limed Tank 9
Removed by crops and drainage	1462.6	1679.3
Added in manure and rainfall	682.2	683.2
Difference, or expected loss	779.4	996.1
Actual gain shown by analysis	37.2	73.0
Sum, or total gain	816.6	1069.1
Average annual gain	54.4	71.3

The total amount of nitrogen fixed by the symbiotic organisms was doubtless larger than that shown by the figures given above for in addition to the removal of nitrogen from the soil in crops and drainage water there was in all probability the unexplained losses whose existence has previously been discussed. These losses have been more than made good by fixation. The estimate is, therefore, probably less in amount than the nitrogen actually fixed.

Planting of the non-legumes was at the same rate where non-legumes were grown alone and when mixed with legumes—the rate being the customary one for these crops in this region. The legumes were entirely supplementary. Their effect was to conserve the original supply of soil nitrogen and to increase total production of crops, usually without greatly curtailing the yields of the non-legumes. This was accomplished in spite of a larger loss of nitrogen in the drainage water from the soil growing the mixed cultures.

The yield of legumes was larger on the limed soil than on the unlimed. With this more abundant growth of legumes has occurred greater fixation. The annual average figure for the unlimed soil being slightly more than 50 lb. per acre as compared with about 70 on the limed.

## MIXED CULTURES OF LEGUMES AND NON-LEGUMES

During the first rotation period no legumes were grown until timothy was sown at which time red clover was sown in the wheat on Tanks 5 and 9.

When the second rotation period began a legume was planted with the cereals on Tanks 5 and 9 and this practice was continued with each of the cereal crops during the second and third rotation periods. Soybeans were planted with maize, Canada field peas with oats, hairy vetch with wheat, and red clover with timothy. At harvest the legumes and non-legumes were separated, weighed, and analyzed. In Table 3 are set down the yields of nitrogen in each crop of non-legume grown alone and for the

TABLE 3.—Yield of nitrogen in non-legumes when grown alone and when in association with legumes<sup>1</sup>

(Pounds to the acre)

Year	Crop	Not limed		Limed	
		Non-legumes alone, Tank 3	With legumes, Tank 5	Non-legumes alone, Tank 7	With legumes, Tank 9
1915	Maize	52.4	44.5	53.7	60.0
	Soybeans		14.9		26.3
1916	Oats	62.3	56.4	51.9	53.9
	Peas		58.5		81.3
1917	Barley	44.0	52.7	51.9	55.1
	Vetch		Killed		Killed
1919	Timothy	51.4	49.9	55.1	16.5
	Clover		42.1		80.6
1920	Maize	42.7	36.3	69.6	66.0
	Soybeans		71.2		103.8
1921	Oats	61.7	52.5	51.8	47.5
	Peas		17.6		53.3
1922	Wheat	51.1	52.0	46.6	50.5
	Clover		No growth		No growth
1923	Timothy	47.2	44.6	29.4	28.6
			56.3		86.4
1924	Timothy	23.1	44.8	11.6	29.8
	Clover		32.9		60.4
Total for non-legumes		435.9	433.7	421.6	407.9
Total for legumes and non-legumes			727.2		900.0
Average annual yield of non-legumes		48.4	48.2	46.8	45.3

<sup>1</sup> Editor's Note:—This table had instructions to set in bold type the figures underscored with a red line, but figures were not so indicated.

non-legume when grown with a legume, also the yields of nitrogen in the legumes, for each year 1915 to 1924 inclusive.

A very striking feature of Table 3 is the large quantities of nitrogen contained in the non-legume crops which were grown in association with legumes. In spite of the space occupied by the legumes and the nutrient they remove from the soil the quantity of nitrogen is often as great in the non-legumes grown with legumes as in those grown alone.

The number of cases in which the non-legumes grown alone yielded more nitrogen was ten as compared with eight cases in which the non-legume grown with the legume yielded more. The average yield of nitrogen per acre was 48.4 lb. for the non-legumes grown alone and 48.2 for the non-legumes in mixed cultures on the unlimed soil and 46.8 for the non-legumes grown alone and 45.3 for the non-legumes when grown with legumes. It must be remarked, however, that the average yields of the non-legumes grown in association with legumes has been favored by the poor growth of the legume that was winter killed or failed to make a good growth. This was the case with the barley in 1919 and the wheat in 1922. This is partly because there was little competition from the legume in these years and also because the manurial effect of the former legume crop was probably still operative.

In the 15 years during which the experiments were conducted there has been a marked difference between the rotations with and without legumes with respect to their tendency to accumulate nitrogen as time goes on. In Table 4 the quantities of nitrogen contained in the crops grown during an entire rotation are stated for rotations with and without legumes.

It will be noticed that the more favorable the conditions for legumes the less the tendency for the yield of nitrogen in the combined crops to decrease from one period to the next. This is apparent when the yields on the unlimed soil are compared with those on the limed. For instance, on the unlimed soil the increased yield of nitrogen due to legumes was not as rapid from one 5-year period to another as it was on the limed soil. This may be seen by comparing line 3 with line 6 in Table 4.

TABLE 4.—Nitrogen contained in crops, growth with and without legumes  
(Pounds to the acre)

	Liming treat- ments	1910-1914	1915-1919	1920-1924	Total
Rotation without legumes	Not limed	368.6	257.4	225.8	851.8
Do with Do	Do	378.3	531.1	457.9	1367.3
Increase due to legumes		9.7	273.7	232.1	515.5
Do without Do	Limed	342.0	260.3	225.3	827.6
Do with Do		385.0	597.4	569.5	1551.9
Increase due to legumes		43.0	337.1	344.2	724.3

## THE DISAPPEARANCE OF NITRATE NITROGEN IN CROPPED SOIL

Several persons, including the writers of this paper, have observed in soil on which crops were growing a disappearance of nitrate nitrogen that could not be accounted for by absorption by the growing plants. The same phenomenon may be seen in these experiments when nitrate nitrogen in the drainage water of tanks without vegetation is compared in amount with nitrogen in crops and drainage combined from the same tanks when in other years they have plants growing on them. If the assumption is made that all the nitrogen is absorbed by plants in the form of nitrate then, when the nitrogen in the crops is added to the nitrate nitrogen in the drainage water for the period during which the soil was cropped, and this is compared with the nitrate nitrogen in the drainage water for the period when the soil was kept bare, it is possible to ascertain the relative quantities of nitrate nitrogen in the soil when cropped and when bare.

Tanks 4 and 8 were kept bare of vegetation during the first 10 years of the experiment and were in crops the last 5 years. Tanks 6 and 10 were in crops the first 10 years and kept bare the last 5 years. When bare of vegetation the surface of the soil was scraped from time to time during the summer to prevent the growth of vegetation, except in years when the corresponding tanks were planted to maize in which years they received the same stirring as did the maize tanks. In the spring the bare soil was spaded in the same way and at the same time as was done for preparing a seed bed in the soil to be planted.

In Table 5 the figures are given for the average annual removal of nitrogen in the crops plus the nitrate nitrogen in the drainage water during the period when the soil of each of Tanks 4 and 8, and 6 and 10 was in crops and the nitrate nitrogen in the drainage water during the period when they were kept bare.

*TABLE 5.—Average annual removal of nitrogen in crops and drainage water from soil when cropped and in drainage water; when bare*

(Pounds to the acre)

Tank	When bare	When cropped
4	82.2	35.8
8	56.0	34.7
6	60.3	56.8
10	71.7	57.1

In the case of each tank the removal of nitrogen was greater during the period when the soil was bare of vegetation than when it was cropped. Since the bare period for Tanks 4 and 8 was during the first 10 years of the



experiment and the corresponding period for Tanks 6 and 10 during the last 5 years of the experiment the effect of season could not be an important factor—neither could the rate of percolation.

The phenomenon and its probable causes have been investigated and discussed in several papers prepared by the writers. They attribute the depressing effect of living plants on nitrate accumulation in soil to the liberation of highly carbonaceous organic matter by plant roots. This organic matter furnishes energizing material to soil organisms which decompose nitrates, using the nitrogen for their growth, and thus decreasing the supply available for the higher plants. These microorganisms are like weeds in their competition with crops for plant nutrients.

### SUMMARY

Certain phases of nitrogen economy in Dunkirk silty clay loam has been studied by means of lysimeters each holding  $3\frac{1}{2}$  tons of soil. Through a period of 15 to 17 years records have been kept of the quantities of nitrogen removed in the crops grown and the drainage water passing through the soil, also of the additions in manure and rainfall. Analyses of the soil were made at the time the lysimeters were filled and at the end of the 15 or 17 years periods and the gain or loss of nitrogen determined in that way.

Two lysimeter tanks were planted to a five year crop rotation of cereals and hay without legumes, two with a rotation including legumes, two were kept bare of vegetation for the first 10 years and cropped without legumes for the next 5 years, and two were cropped for the first 5 years and kept bare for 10 years. One of each of these pairs of tanks was limed from time to time while the other received no lime.

The quantity of nitrogen removed in crops and drainage combined was in every case more than that added in manure and rainfall. The difference between these two items might be expected to represent the total loss of nitrogen from the soil. The loss was also found by computing the difference between the quantity of nitrogen in the soil at the beginning and end of the experiment.

The loss as determined in these two ways agreed quite well for the two tanks growing a crop rotation of non-legumes during the entire period covered by the experiment. In the soil of the two tanks kept bare of vegetation for the first 10 years the loss found by analysis was more than 300 lb. greater than the loss found by subtracting the nitrogen applied in the manure and rainfall from that removed in the crops and drainage. This indicates that there is some way other than those mentioned by which nitrogen escapes from soil under the conditions maintained in the bare tanks. It is further suggested that the presence of living plants or of plant residues is concerned in some way with the conservation or acquisition of the soil nitrogen not removed in crops or drainage water.

Soil in the two tanks having a rotation of legumes and non-legumes.

mixed showed a very large difference between the quantity of nitrogen removed in the crops and drainage and that contained in the manure and rainfall. On the other hand the analyses showed an actual gain in nitrogen. The sum of these two may be taken to represent the amount of fixation, most of which must be due to the symbiotic bacteria. This amounted to about 50 lb. annually in the unlimed soil and about 70 lb. in the limed.

A striking feature in the growth of the legume and non-legume mixtures is the large quantities of nitrogen contained in the non-legume crops. The number of cases in which the non-legume grown alone yielded more nitrogen was ten as compared with eight in which the non-legume grown with the legume yielded more. The average yield of nitrogen per acre was 47.6 lb. for the non-legume grown alone and 46.7 for the non-legume in mixed cultures. In addition to this the legumes produced an average of 90.4 lb. of nitrogen making a total of 137.1 lb. of nitrogen per acre as the average annual yield of nitrogen from the mixed cultures.

The yields of the mixed cultures showed less tendency to decrease from one 5-year period to another than did the cultures of non-legumes alone.

There was a disappearance of nitrate nitrogen from soil growing non-leguminous plants that could not be accounted for by the nitrogen absorbed by the plants. It was indicated by the fact that the nitrate nitrogen in the drainage water from bare soil was larger in amount than the nitrogen in the crops and drainage water combined from the same soil when producing crops. This depression of nitrate nitrogen accumulation has previously been ascribed by the authors to the liberation of organic matter low in nitrogen by the roots of living plants which serves as energizing material for bacteria which decompose nitrates. The data obtained from this experiment are in line with results obtained previously.

#### LITERATURE CITED

- (1) Russell, E. J., and Richards, E. H. 1920. The washing out of nitrates by drainage water from uncropped and unmanured land. *Jour. Agr. Sci. [England]* 10: 22.
- (2) Shutt, F. T. 1925. Influence of grain growing on the nitrogen and organic matter content of the western prairie soils of Canada. *Dominion Experimental Farms, Bul.* 44, n. s.

# HOW PLANTS FEED

E. TRUOG

*University of Wisconsin, U. S. A.*

## INTRODUCTION

Chemical analysis of plants indicates that every element which is found in a soil is also found in plants that grow on this soil. This indicates that the cell walls and membranes of the root hairs allow the inward diffusion or osmosis of practically everything that is dissolved in the soil solution. The soil solution, however, usually does not contain a sufficient amount of all of the essential elements, so that plants can satisfy their needs in all respects by simply "drinking in" the soil solution. It is believed by some that plants feed entirely on the soil solution. As regards the feeding for nitrates and sulfates this belief is undoubtedly true, but as regards the feeding for other essential soil constituents it is probably far from true.

## SOLID PHASE FEEDING

For example, 1 kg. of dry corn fodder contains 2 g. of phosphorus and requires 300 kg. of water for its growth. If all of this phosphorus were to be carried to the root hairs by this 300 kg. of water, it would mean that this water when it existed as the soil solution would have to contain nearly 7 p.p.m. of phosphorus. This is very much more phosphorus than is usually contained in the soil solution of soils in the humid region on which corn grows luxuriantly. In fact some soils contain less than 1 p.p.m. of phosphorus in the soil solution and yet they produce good crops of corn.

Undoubtedly plants usually obtain a large portion of their potassium, calcium and magnesium directly from the soil solution. In the arid region the soil solution is probably often concentrated enough in all of the essential constituents so that plants can get their entire requirements directly from this source. Plants, however, are fortified, as we shall see, so that they are not dependent on the soil solution for their requirements of the mineral elements.

In support of the idea that plants feed entirely on the soil solution it might be argued that by means of diffusion, phosphates and other constituents moved through the soil solution to the root hairs to be absorbed independently of the movement and absorption of water and thus make up the apparent deficiency. Movement by diffusion of soluble constituents in the soil, however, has been shown to be so extremely slow that it is not a factor of importance in this connection.

One is thus led to the conclusion that plants in some way directly attack the solid mineral constituents of the soil and bring essential elements into solution so that they may be absorbed independently of the intervention of the soil solution. The remarkable manner in which root hairs cling to soil particles suggests that there must be some important relation between this phenomenon and plant feeding.

How root hairs feed on these particles with which they are in immediate contact, may be beautifully illustrated in the following way: An artificial root hair is made by filling a collodion sack shaped like a test tube with dilute hydrochloric acid. A crystal of calcite is made to cling to the sack by attaching it with a rubber band. Instantly the crystal starts to dissolve as is shown by vigorous effervescence of carbonic acid, and the calcium chloride formed diffuses rapidly into the interior of the sack where a positive test for calcium can be secured within the course of a minute or two. In time the entire crystal disappears and the calcium enters the sack without any liquid having been furnished from without the sack.

The experiment just described indicates how plants may feed on solid constituents independently of the soil solution, and for the convenience of the present discussion this type of feeding will be referred to as solid phase feeding, in contrast to feeding on the soil solution which will be referred to as liquid phase feeding. The extent of solid phase feeding in any particular case may be dependent on the deficiencies of the soil solution. For example, if the soil solution is deficient in phosphorus, the plant may have a mechanism by means of which the solid phase feeding for phosphorus is increased in order at least partly to compensate for the deficiency. This might be accomplished through the action of chemotropism on the root hairs.

The influence of chemotropism on the root hairs would, if it exists, be greatest for the elements that are deficient, for, if an element existed in abundance in the soil solution chemotropism as regards this element would be nearly equal in all directions and hence the net result would be practically zero. If, however, an element did not exist in abundance in the soil solution, its concentration would be appreciably greater on the sides of the root hairs towards the soil particles that are the source of this element. Chemotropism could thus bring a high proportion of the root hairs to the soil particles containing the deficient element. After the root hairs strike the particles, chemotropism may further cause the root hairs to grow into a most intimate contact with the particles, that is, actually grow in such form as to fit into the depressions and around the elevations. This is actually what takes place and it is stated by some writers that the root hairs make a growth fusion with the soil particles.

The area of contact or fusion between a root hair and a soil particle becomes to a certain extent a system within itself, closed to a large extent

to outside influences especially of the soil solution. This provides an ideal situation for promoting solid phase feeding.

Comber (4) quite recently has pointed out the inadequacy of the theory that plants are dependent entirely on the soil solution for the essential soil elements, and has emphasized the importance of the close union between root hairs and soil particles in plant feeding. The writer does not, however, favor his view that plants may absorb the soil nutrients in colloidal form or that the soil particles are dissolved to a notable extent at the points of contact by organic acids and other organic compounds. The writer believes that the only agents of general importance in this connection are water and carbonic acid, and that in the main only material in true solution enters the plant:

The experiments of Czapek some years ago and also those of later investigators indicate that agricultural plants do not excrete other acids than carbonic in notable amounts. Carbonic acid, however, under certain conditions is a fairly vigorous agent. Water saturated with it has a pH of about 4.

The carbonic acid excreted in the area of contact between the root hairs and soil particles is prevented from escaping rapidly by the closeness of contact, and due to the small amount of water involved a saturated solution is quickly produced. The almost instantaneous removal by the root hairs of the soluble products formed in the action of the carbonic acid on the soil particles makes it possible for the carbonic acid to act under maximum efficiency. The conditions for the action of carbonic acid in solid phase feeding are thus usually ideal and hence the carbonic acid acts much more vigorously than under ordinary conditions that we are accustomed to.

In feeding on the colloidal-complex for exchangeable base the carbonic acid furnishes the cation, hydrogen, which has a maximum replacing power and hence calcium, magnesium, potassium and even ammonium are easily brought into solution at the points of contact or fusion as carbonates and bicarbonates to be used by the plant. This also explains how plants feeding on acid soils are able to secure salts as carbonates which may be used partly for neutralization of acids and regulation of reaction in their own system.

The area of contact or fusion between root hair and soil particle being to a certain extent a system within itself and closed largely to outside influences of the soil solution explains how plants may secure the required traces of iron even in neutral and calcareous soils. In the areas of contact between root hairs and iron minerals, the pH may be near 4 due to carbonic acid. At this reaction a mere trace of acetic or other organic acid probably suffices to dissolve the minute amount of iron needed to supply the plant even though the soil solution in general has a pH of 7 or more.

Plants vary greatly in their feeding power as has been shown by Merrill (7), Prianischnikov (9), Kossowitsch (5), Truog (10) and others, and hence any theory of plant feeding must, in order to be valid, explain these differences. For example, in quartz cultures buckwheat and sweet clover feed strongly on raw rock phosphate while oats and corn do not. The explanation is easily found in the law of mass action. The action of carbonic acid on raw rock phosphate produces two soluble products and may be written as follows:



If both products of this reaction are absorbed equally by the root hairs, the reaction continues rapidly and indefinitely and the plant is able to feed strongly on the raw phosphate. This is actually the case with buckwheat and sweet clover which require much calcium. Oats and corn require much less calcium and, hence, when they feed on raw phosphate calcium bicarbonate soon accumulates to the point of saturation in the region of solid phase feeding which prevents further action of the carbonic acid on the phosphate. As a result oats and corn are weak feeders on raw rock phosphate. In general, then, it has been found (10) that plants with a high calcium content feed strongly on raw rock phosphate in quartz cultures, while plants with a low calcium content are weak feeders under similar conditions:

Due to the fact that it does not take much calcium bicarbonate to form a saturated solution, the reaction between carbonic acid and raw phosphate soon comes to a standstill if the calcium bicarbonate is not removed. If plants excreted acetic, citric or malic acid, a very soluble calcium salt would be formed and the reaction would continue quite long even though the calcium salt was not entirely removed. Since the reaction apparently slows up quickly in solid phase feeding when the calcium salt is not fully removed there is good indirect evidence here that plants do not excrete other acids in notable amounts than carbonic.

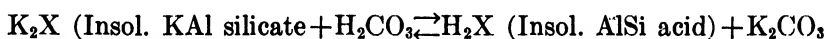
Further evidence in support of the proposed theory is furnished by the work of Bauer (1) who grew corn on raw rock phosphate in quartz cultures and gave some of the cultures an occasional leaching with water. As we should expect, on the basis of the theory, the corn on the leached cultures grew better because the excess of calcium bicarbonate was removed which allowed the solvent action of carbonic acid on the raw phosphate to continue.

It is well known that the use of ammonium sulfate or nitrate in quartz cultures greatly aids the utilization of raw phosphate. This is probably due to two reasons: First, both of these salts give rise to an acid condition which aids directly in making the phosphate available. Second, the presence of these salts increases the solubility of calcium bicarbonate which makes it possible for the action of carbonic acid on raw phosphate

to continue farther even though the calcium bicarbonate is not all removed by plant feeding.

Plants with a low calcium content feeding on rock phosphate in acid soils, may be aided by the capacity of the acid soil to take up the calcium bicarbonate. Because of this situation plants like oats and corn feed much more strongly on rock phosphate in acid soil cultures than in quartz cultures. Field experiments also show a higher availability of rock phosphate in acid soils than in the non-acid ones. All of this conforms with the theory.

The feeding on raw rock phosphate is peculiar in that it involves the formation of two soluble products. In practically all other cases of solid phase feeding only one soluble product is formed. In the feeding on the relatively insoluble silicates for the various bases only one soluble product is formed in each particular case as follows:

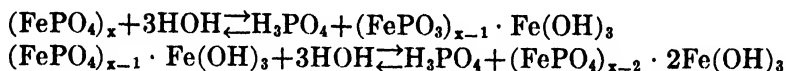


The ability to feed on these materials is therefore dependent on different factors than is the case with raw rock phosphate. In fact buckwheat which is one of the strongest feeders on rock phosphate is one of the weakest feeders for potassium in feldspar. Sweet clover feeds strongly on both rock phosphate and feldspar. What outstanding characteristics in relation to feeding are similar and dissimilar in these two species of plants? Both have high calcium contents and that explains their common ability to feed strongly on rock phosphate. The sap of the sweet clover roots and tops is nearly neutral, while in the case of buckwheat it is very acid having a pH of 4 to 5. It is possible that the extremely acid condition of the membrane of the buckwheat root hairs greatly lessens their permeability to the passage of potassium carbonate into the plant and transference to the place where needed. It is also possible that the high acidity of the plant requires a higher concentration of potassium ions in the plant in order to be effective. Alfalfa and corn are given as additional examples. Alfalfa has a very slightly acid sap and behaves like sweet clover, while corn has a strongly acid sap and behaves like buckwheat. This phase of the subject needs much further investigation before definite conclusions can be drawn.

The much greater feeding power of sweet clover than buckwheat for the potassium of feldspar furnishes additional evidence that other acids than carbonic are not excreted by these plants for if other acids than carbonic were involved, a much more acid excretion would be given off by the buckwheat since it has a much more acid sap, and as a consequence the buckwheat should then feed more strongly on the feldspar than the sweet clover.

Freshly precipitated iron and aluminum phosphates are well known (5) to be good sources of phosphorus for most all species of plants. The

reason for this is that they readily hydrolyze probably in the following way:



Since only one soluble product is formed and this through the action of water, it is apparent why fresh ferric phosphate is readily available to different plants. As the hydrolysis proceeds the resulting phosphate becomes more and more basic and less easily further hydrolyzed so as to liberate more phosphorus. In quartz cultures where only one or two crops are grown and a considerable excess of the ferric phosphate is used there is not removed a sufficient portion of the phosphorus to allow the formation of a very basic phosphate. As a result there is a good supply of available phosphorus. Under soil conditions where cropping proceeds indefinitely there is time for the formation of a phosphate that is so basic as to be a very poor source of phosphorus for plants. Because of this it is better to keep the phosphorus in the form of calcium phosphate which does not become more and more basic as plant feeding takes place, since the calcium is removed as the soluble bicarbonate.

What has been said of ferric phosphate holds for aluminum phosphate.

#### RELATION OF CARBONIC ACID AND OTHER ACIDS TO FEEDING POWER OF PLANTS.

It has been suggested by some that differences in amount of carbon dioxide excreted by plant roots may account for differences in feeding power. In extensive experiments, Parker (8) and Kossowitsch (6) found very little relation in this respect. It appears that the efficiency of use of the carbon dioxide that is excreted is much more important than the amount excreted.

Extent of root system is undoubtedly a very important factor in plant feeding. All other things being equal, feeding power should be proportional to extent of root system. Other factors, however, are usually not equal and hence we may have two plants like corn and alfalfa both with extensive root systems but with radically different feeding powers. Differences in extent of root systems, therefore, do not explain the marked differences in feeding power of different species of plants.

A study of the conditions involved pertaining to the best interests of the plant seems to forbid the excretion of other acids than carbonic. If other acids were generally excreted what would limit the amount? In case of excessive leaching would not the removal of the acid cause the excretion of so much acid as to greatly disturb the internal reaction and osmotic condition of the plant? If acids like acetic and citric were excreted, what would prevent the excretion of salts of essential elements? Experimental data seem to indicate that with the exception of water and car-



bonic acid and under normal conditions no more than traces of the salts or other substances, when once inside of the common agricultural plants, ever return to the soil through the living roots. In other words very little exosmosis of salts takes place in the roots.\*

The arguments against the belief that other acids than carbonic are excreted in notable amounts by plant roots may be summarized as follows:

(a) Direct chemical tests for the presence of appreciable amounts of excreted acids have given negative results.

(b) Differences in the feeding power of different plants for raw rock phosphate are in accordance with the contention that only carbonic acid is excreted.

(c) The much stronger feeding power of sweet clover than buckwheat for feldspar is not in accordance with the contention that other acids than carbonic are excreted and are a factor, for if they were, then buckwheat with a very much stronger acid sap should excrete a much stronger acid and hence feed more strongly on the feldspar.

(d) The excretion of some other acid than carbonic would not explain the observed differences in feeding power.

(e) General excretion of other acids than carbonic would probably be fatal to the plant.

### • ELECTRICAL THEORIES OF PLANT FEEDING

An electrical theory of plant feeding was advanced by Casale (3) in 1921, and more recently Breazeale (2) has advanced one along somewhat similar lines. Casale holds that the colloids of the soil and root hairs become cemented together into one colloidal complex. The colloids of the plant throw off hydrogen ions and become negatively charged, but less so than the soil colloids. This results in a potential difference between plant and soil and a migration of cations from the soil to the plant. By a similar process the cations pass on and circulate in the plant. The greater the potential difference between the plant and the colloids of the soil the more intense is absorption. Casale's theory does not explain the absorption of anions.

Breazeale holds that a demand for an element arises in the tissue of a plant and is carried to the absorbing surface of the root by means of an unsaturated carbon compound bearing a plus or a minus charge. In the case of potassium the protoplasm in the leaf may remove an atom of potassium from a colloidal compound and use it to build a permanent compound. This removal leaves the colloidal compound out of equilibrium and with a minus charge. This charge is transmitted by replacement successively through the cells to the root tip and there appears as a minus charge which attracts from a salt the potassium with a plus charge. In a similar way, a demand for  $\text{NO}_3$  might originate and be

satisfied. In this way a demand for an element might be transmitted a considerable distance through the soil solution.

The writer does not accept the electrical theory of plant feeding for several reasons. In the first place, the theory requires that a very peculiar process or phenomenon takes place, the like of which is practically unknown in the laboratory or elsewhere. This sort of a process surely does not take place in animal nutrition, and it seems reasonable to believe that the nutrition of the living cells is much the same in animals and plants.

In the second place, the theory does not explain the presence of large amounts of mineral matter in plants which apparently serves no use but simply entered because it existed in the soil solution. In fact, all the mineral elements found dissolved in the soil solution are found in plants and often in large amounts, even though they be unessential. It is reported that the ash of plants may contain from 1 to 13 per cent of zinc; 2 to 27 per cent of aluminum; 7 to 14 per cent of manganese; and 1 per cent of copper. A theory of plant feeding in order to be valid must explain the presence of these.

Furthermore, the electrical theory does not offer a good explanation of the differences in the feeding power of plants.

### SUMMARY

Plants are not dependent on the soil solution for their supply of the essential mineral elements. They are so fortified that if the soil solution is not sufficiently concentrated in one or more of these elements they can attack the soil particles and thus partially or wholly make up the deficiency.

The close union which root hairs make with the soil particles provides ideal conditions for solution and absorption. Each place of union or contact is to a certain extent a system within itself, and is not readily disturbed by the reaction of the soil solution. This makes it possible for plants to secure iron even in calcareous soils.

All of the evidence both direct and indirect points to the conclusion that carbonic acid is the only acid excreted in notable amounts by agricultural plants.

The carbonic acid excreted is capable of producing a pH of 4 at the point of contact between root hair and soil particle and is thus an active agent in liberating replaceable bases and in dissolving calcium phosphate.

Differences in the feeding power of common agricultural plants for the essential elements of comparatively insoluble minerals are not due primarily to differences in amounts or kinds of acids excreted. The differences are due to several factors, some of which are concerned with external equilibrium conditions around the feeding roots, and others with permeability of root hairs and equilibrium conditions inside the plant where the

elements are actually used. Extent of root system is a factor but not a controlling one. \*

, In case two soluble products are formed in the feeding region of the roots due to the action of carbonic acid on a mineral as is the case with rock phosphate, the feeding power follows the law of mass action and chemical equilibrium, being dependent on the removal of both of the soluble products either by the plant or partly by the plant and partly in other ways; thus plants with a high content of calcium feed strongly on rock phosphate even in quartz cultures because they remove both the soluble phosphate and soluble calcium bicarbonate. ^

#### LITERATURE CITED

- (1) Bauer. 1920. Soil Sci. 9: 235.
- (2) Breazeale. 1923. Jour. Agr. Research [U. S.] 24: 41.
- (3) Casale. 1921. Staz. sper. agr. ital., 54: 65 Abs. in Chem. Abs., 16: 604.
- (4) Comber. 1922. Jour. Agr. Sci. [England] 12: 363.
- (5) Kossowitsch. 1900-9. Russ. Jr. Expt. Landw., 1: 657; 2: 730; 3: 145; 5: 598; 10: 839.
- (6) ———. 1904-6. Ibid. 5: 943; 7: 251.
- (7) Merrill. 1898. Maine Agr. Expt. Sta. Ann. Rept. 65.
- (8) Parker. 1924. Soil Sci. 17: 229.
- (9) Prianischnikov, 1902-7. Landw. Vers. Sta., 56: 107; 65: 23; 75: 357 and 372.
- (10) Truog. 1912-16. Wisc. Agr. Expt. Sta. Res. Bul., 20 and 41.
- (11) ———. 1915-22. Science, 41: 616; 56: 294.

# PRODUCTIVITY OF PEAT SOIL AS INFLUENCED BY HEIGHT OF THE GROUND WATER TABLE <sup>1</sup>

H. B. ROE

*Minnesota Agricultural Experiment Station, U. S. A.*

## INTRODUCTION

*This Discussion Based on Results of a Definite Experimental Project.*—During the last decade the agricultural development of peat lands has become a very live issue in the United States and especially so in the Lake States of Michigan, Wisconsin, and Minnesota, the total area of peat in these three being about 12,000,000 acres of which Minnesota alone contains about 7,000,000 acres or approximately one-eighth of the area of the state. A large part of this area probably is capable of agricultural development. The demand for reliable information as to proper methods of farming peat lands, therefore, became so insistent that for the past eight or ten years the Minnesota Agricultural Experiment Station has carried on extensive investigations along this line for the most part under the leadership of Dr. F. J. Alway our well-known Chief in Soils.

## DRAINAGE INVESTIGATIONS PROVIDED FOR

*Drainage a Prime Requisite.*—As drainage is a prime requirement in the agricultural use of peat soils the drainage staff was early called to assist in the problem. It was at the direct suggestion of Dr. Alway to the writer in the fall of 1919 that the experimental project on which this paper is based was planned in the late fall of 1921.

*Statement and Purpose of the Project.*—The purpose of the project was, by actual cropping of plots on which the ground water table was artificially controlled—at least theoretically—at different specific depths, to determine what depth of water table produced the best results in the growing of the standard field crops and vegetable crops, and to study the movement of ground water in peat soil, as reliable information along these two lines is necessary to the intelligent design of tile drainage systems for peat soils.

*Location of the Experimental Tract.*—A piece of typical tamarack swamp—in a high lime peat bog—was located in a newly established drainage district in Hennepin County, Minnesota, in the heavily glaciated

<sup>1</sup> Journal Series Paper No. 677 of the Minnesota Agricultural Experiment Station, written for the Committee on Soil Technology of the First International Congress of Soil Science, meeting at Washington, D. C., U. S. A., June 13 to 22, 1927.

district a few miles outside of the present basin of Lake Minnetonka, where, at the outset, it appeared possible to secure a control of the ground water on consecutive plots at average depths below the surface of 1, 2, 3, 4, 5, and 6 feet, respectively. However, although this tract was cleared, broken and put under cultivation in 1922, no data or results of definite value accrued to the Experiment Station, prior to 1925, beyond the clearing and breaking of the land, its thorough tillage, seasonal packing of the surface, the installation of the subirrigation and control works, and the completion of the settlement of the surface so characteristic of peat land under the influence of drainage and tillage.

*Beginnings, Organization and Cooperation.*—The project was reorganized in the early spring of 1925 under the leadership of the writer. Under this reorganization the work has been carried on in cooperation with the Division of Agronomy and the Division of Horticulture. The Division of Agronomy assumed charge of the preparation of the seed bed, provided the fertilizer for the entire tract and assumed the responsibility for the planting, care, harvesting, and securing of records of the field crops. The Division of Horticulture assumed a similar responsibility for the vegetable crops. The Division of Agricultural Engineering assumed the responsibility for the design, installation and care of all engineering features, the maintenance and recording of the water levels and the securing of rainfall and temperature records.

About one-fifth of the tract was made available for independent investigations by the Division of Botany and Plant Pathology. However, this latter work has no further connection with the discussion in this paper although, by mutual agreement, all records of all divisions operating on the tract are mutually available.

## CHARACTER OF THE BOG

*Surface.*—The original surface of the tract, after clearing, was moderately smooth with a fairly uniform fall toward the south as indicated on the profile in Fig. 1. This surface, by 1925, had become permanently depressed as indicated in the same figure, thus destroying the possibility of attaining a 6 foot depth of water table and even interfering with the 4 and 5 foot controls to a serious extent.

*Soil.*—The peat soil was, originally, a raw, coarse, fibrous peat but the three years tillage from 1922 to 1925 had reduced its surface, to plough depth at least, to a firm, finely divided, well decomposed condition.

*Subsoil Conditions.*—For the most part the peat was so deep that the character of the subsoil had no visible surface influence but about the east two-thirds of the east half of the one foot control area (see fig. 1) was underlain by a medium fine white quartz sand at a depth of  $2\frac{1}{2}$  to 3 feet. This sand bed also extended southward under the two foot control still sufficiently near the surface, under the east half, to seriously hamper the

control of the ground water table at the two foot depth. It did not seem to be near enough the surface, farther south, to seriously influence the water control on the other levels.

### SOURCE OF WATER SUPPLY

The source of the water supply was a large tile submain of the county ditch which, throughout the year, carries ample water to insure, at all times, a supply which can be regulated by a dam within the take-off well at the tile main.

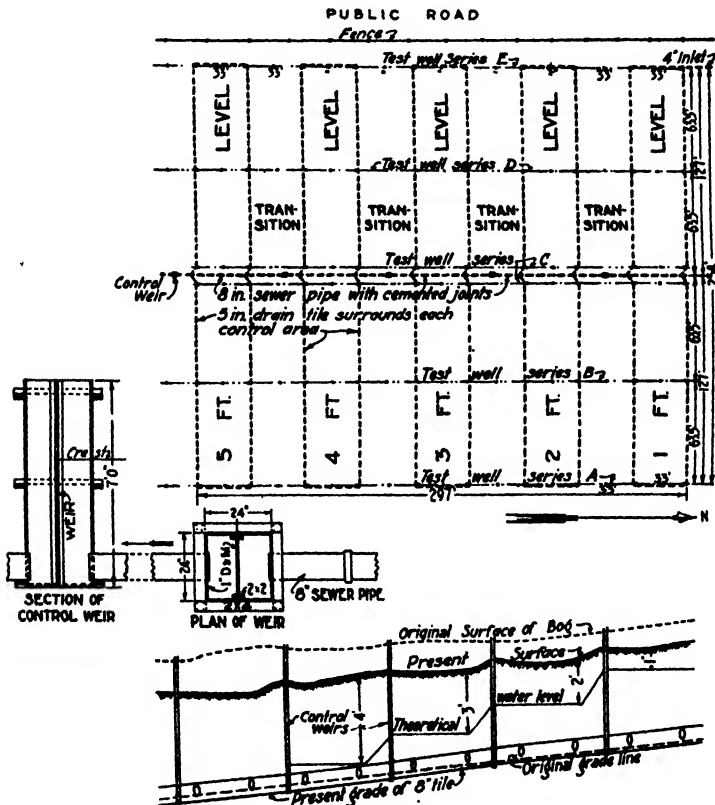


FIGURE 1.—General layout of experimental tract

### GENERAL LAYOUT OF EXPERIMENTAL PLOTS, AND DRAINAGE AND CONTROL WORKS.

*The Ground Plan.*—The general layout was as shown in profile and plan in Fig. 1. The water flow is from north to south as the north end is the high end. The one foot control plot, that is, the plot under which the water table is held one foot below the surface, extends across the north end, followed by a transition plot, and each successive control plot, separated from the next control by a transition plot, follows in consecutive

order going toward the south. Each control plot and each transition plot is 33 feet wide north and south and 127 feet long east and west on each side of the center line or main tile.

*Design of Sub-irrigation System.*—The main tile is 8 inch sewer pipe with tightly cemented joints but opening free into each control weir box. The original grade of the main tile was uniformly 0.25 per cent but the settlement of the tile in the soft bog has made the final grade somewhat irregular as shown in the profile in Fig. 1. However it is still sufficient to carry the water with ample velocity. The original depth of the base of the main tile, below the bog surface, was about  $5\frac{1}{2}$  to 6 feet. In the original plan, each control plot was surrounded by a loop of 5 inch drain tile laid with rather open joints at depths of from 4.8 to 6.4 feet according to the control plot, and with a decided down grade toward the main on each loop.

*Better Control on the One Foot Level Sought in 1926.*—Experience during the growing season of 1925 plainly seemed to indicate that the sand subsoil before mentioned, under the northeast corner, robbed the water from the sub-irrigation loops of tile on the 1 and 2 foot controls to such an extent as to make it impossible to maintain the desired elevation of the ground water table on these plots east of the main. Hence, at the beginning of the season of 1926, in the hope of offsetting the influence of the sand deposit the old loops of tile around these two plots were plugged and new loops laid from 2 to  $2\frac{1}{2}$  feet deep but not tapping the sand deposit. As the original surface at the extreme northeast corner was noticeably higher than the rest of the bog and as there were some extra low spots at the north end of the west half some leveling down of the surface was done in the spring of 1926 in the hope of further offsetting the influence of the sandy subsoil. As may be noted by examining the actual average depths of the water table shown in Tables 1 and 2 and the water curve charts in Figs. 3 and 4, we accomplished very little by this modification, the sand deposit seeming to be too large or too dry to overcome its influence.

*Control Weirs.*—The control of the water levels was effected by means of the weirs, the plan and location of which are shown in Fig. 1. The weir boxes were made of good quality white pine  $\frac{3}{4}$  inch dressed and matched, reinforced by collars of 2 by 4 inches. The weir aprons were of similar material and were removed each fall to lower the water level before freeze up and replaced each spring with new.

*The Inlet.*—The inlet for the water supply was through a 4 inch tile line entering the 5 inch control line at the northwest corner of the tract.

*Test Wells.*—Lines of test wells in which to measure the actual height of the ground water table, were located as shown on Fig. 5 for 1925 and as shown on Fig. 6 for 1926. These test wells were of 4 or 5 inch drain tile and extended to a depth of 5 to 6 feet below the surface. The tops of

TABLE 1.—Comparative yield of field crops on water controlled peat plots for 1925-1926

Item number	Limits of full root control zones	Theoretical depth of water table in feet	Average actual depth of water table for growing season in feet	Corn—Minn. No. 13*				Sugar beets Chaska		Flax—No. Dak. No. 114				Oats—Gopher				Soybean hay Chestnut	
				Ear corn bushels of 70 lb. per acre	Stover tons of 2000 lb. per acre			Tons of 2000 lb. per acre		Grain bu. of 56 lb. per acre	Straw tons of 2000 lb. per acre			Grain bu. of 32 lb. per acre	Straw tons of 2000 lb. per acre			Tons of 2000 lb. per acre	
				1925	1926	1925	1926	1925	1926	1925	1926	1925	1926	1925	1926	1925	1926	1925	1926
1		1.0	0.9	58.8	58.8	1.8	2.1	10.9	14.1	8.1	1.7			11.7	2.0	2.4			
2			1.0	52.5	57.0		2.0				1.6			17.8	1.8				
3			1.1							6.8				18.2					
4			1.2																
5		1.0	1.3	59.1		1.7		8.9	12.3										
6			1.5																
Av. 0.6 to 1.5				55.8	57.9	1.8	2.0	9.9	13.2	7.5	1.7			18.0	1.9	2.1			
7		1.0	1.6					21.0											
8		1.0	1.8				2.9							24.4	2.3	1.9			
9		2.0	2.2																
10			1.9	83.0															
11			2.0																
12			2.1																
13			2.2	82.1	65.7	2.5	2.3			6.9	1.8			22.2	2.1	1.8			
14			2.2	69.1			2.4							19.4					
15			2.3																
16			2.3																
17			2.4	66.5	2.5			21.0	16.3										
18		2.0	2.5	79.8	2.7			20.0	13.8					20.7	2.0	2.4			
Av. 1.6 to 2.5				76.1	72.6	2.6	2.5	20.6	15.1	6.8	1.7			21.3	2.1	2.0			

\* Corn Stover for 1926 is estimated as exact data were lacking.





**TABLE 2.**—*Comparative yield of vegetable crops on water-controlled peat plots for 1925-1926*

[illegible]

Development slightly below 2-4' levels.  
Not uniform in size.

<sup>b</sup> Development below 2-4' levels.  
<sup>c</sup> Height less than on 2-3' levels.

Plants did not thrive.  
Bulbs small.

these wells were approximately at the ground surface and each was capped with a wooden cover held in position by a small block extending down into the well.

### SETTLEMENT OF THE BOG

*Time and Extent of Settlement.*—Bearing out almost universal experience in observation of reclaimed and cultivated peat bogs this tract settled

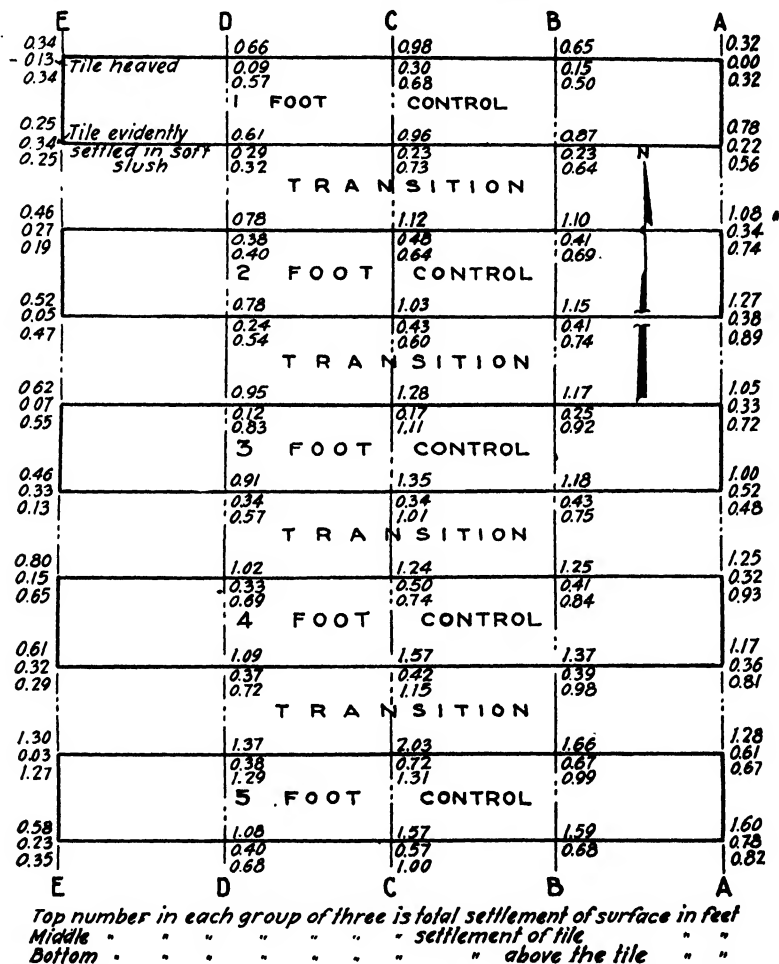


FIGURE 2.—Diagram of water-controlled peat plots, showing settlement of surface from drainage and tillage

measurably after the installation of the tiling system in 1921 but the level records in 1925 and 1926 showed clearly that the settlement was completed before the open season of 1925. The amount of this settlement is indicated in Fig. 2 in which the top number in each group of three indicates the total surface settlement, the middle number the settlement be-

low the tile, and the lower number the settlement above the tile. This last is evidently the most significant and varies from an average of about 0.5 of a foot at the north end to about 0.9 of a foot at the south end, the variation being approximately proportional to the depth of the water table, as might be expected.

### CONTROL OF THE WATER LEVELS

*Method of Measurement.*—The depth of the water table was measured at each test well every Monday and Thursday throughout each season and averages of these measurements were computed for each well, for each month, and also for the 4 month and the 6 month growing seasons. From these averages the average actual depths were also computed for each crop strip on each control and these actual average depths were entered in Tables 1 and 2, each opposite its proper item. In computing these averages, the well-known fact that the ground water curve rises more rapidly near the tile lines than at a distance from them, was disregarded. The final zoning was based on these measurements and averages rather than on the theoretical depth of control. The same method of computing averages was followed during the season of 1926, also, in order to make general results comparable with those secured in 1925. In the middle of the season of 1926, however, additional test wells were installed on the B series and on the D series, as shown in Fig. 6, in order to determine more nearly the true shape of the ground water curve on all plots. In 1926 a new line of test wells was also established 5 feet away from and on each side of the control weir line in the center of the tract, to replace the single line of wells placed just over the main tile in 1925, because it was found in 1925, that the location of the ground water curve along the center as determined from the wells and the weirs just over the main tile was not representative of the ground water curve a short distance away from and on either side of the center line.

*Relative Accuracy of Averages.*—It is evident that the method used in computing the average depth of the water table for any part of the area of any plot, ignoring, as it does the arching of the curve, does not give the true average; but, as the same method was used in both seasons, the results will be relatively comparable as will also, it is assumed, the physical effects on the crop growth, soil and air temperatures, etc.

*Maximum and Minimum Heights.*—Figures 3 and 4 show, for 1925 and for 1926 respectively, the approximate maximum, minimum, and average positions of the water table, along the B and D series of wells. The upper and lower curves are only approximate because the exact maximum or exact minimum depth, naturally enough, did not occur at each well on the same date; hence, there were selected to represent the approximate maximum or approximate minimum curves along these lines, for the season, the readings of that date which showed the depth at a maximum or at a mini-

imum for a majority of the wells on the line. Where it seemed advisable or necessary, in order to show even an approximately correct maximum or minimum for the whole line the date was shifted in the course of the chart as indicated by a footnote on its face. The average curve shown is the average for the entire season, May to October inclusive, as this seemed to cover the growing season for the majority of the crops raised. The four

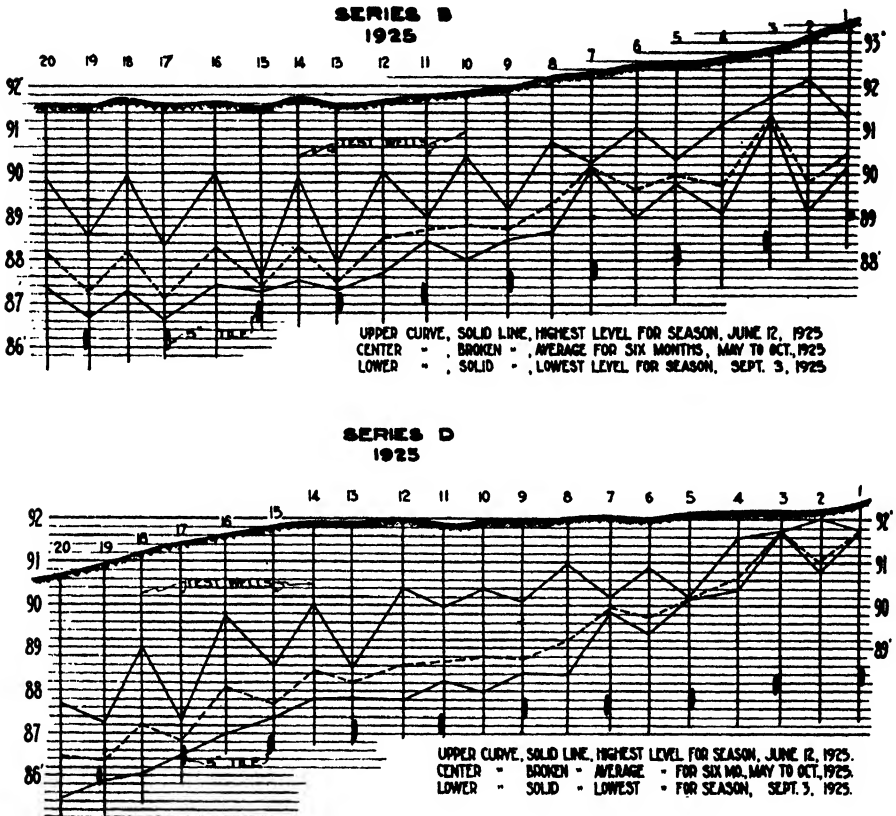


FIGURE 3.—Profiles showing maximum, average, and minimum heights of the ground water table along the B and D series of test wells for the season of 1925

months average, plotted on top of the six months, followed the latter so closely that it served only to dim the clarity of the chart; hence, it was omitted.

*Pertinent Facts Relative to the Water Control.*—The following interesting and pertinent facts become evident from a study of the charts in Figs. 3 and 4 in conjunction with the rainfall record for each season shown in Fig. 7:

1. Exact control of the water table, even within practical limits, is impossible without greatly decreasing the spacing between the

control lines of tile, and such a proceeding would make the cost of the plant prohibitive in practice.

2. Even the average elevation of the peak of the ground water curve will exceed the elevation over the tile lines by from 0.25 of a foot to a foot according to frequency and amount of rainfall and to moisture demands of crops; while the variation, at the peak of the ground water curve, between maximum and minimum elevations will have a range of from 1 to 3 feet, from the same causes.

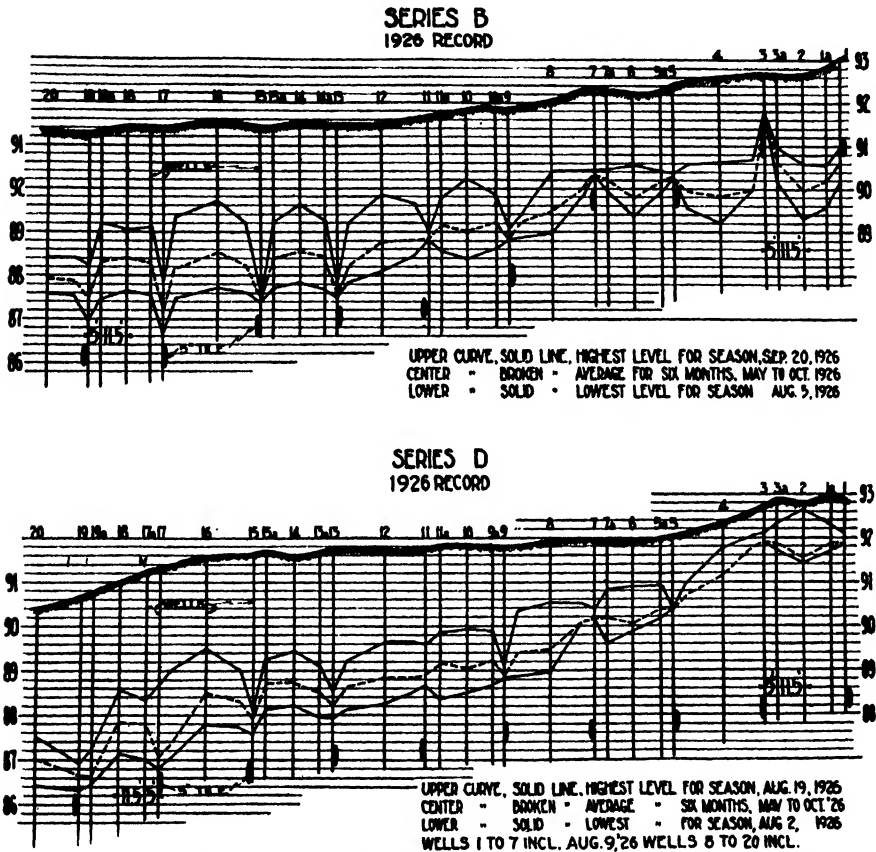


FIGURE 4.—Profiles showing the maximum, average, and minimum heights of the ground water table along the B and D series of test wells for the season of 1926

3. The more exact average depths that would be obtained for any given area by taking into consideration the true shape of the ground water curve will run from 0 to 0.3 of a foot higher than the relative averages as obtained in this project.
4. Subsoil conditions, the physical character of the deeper layers of the peat, the type of crop, its normal rooting depth, its moisture requirements, and the time, amount and character of the rain-

fall all tend to militate against the maintaining of any particular average depth of water table that may be artificially provided for.

5. Maximum heights of the ground water table tend to occur from 1 to 3 days after heavy precipitation, according to the month and the moisture demands of crops.

## FERTILIZATION AND THE CROPPING PLAN

The furnishing and distribution of fertilizer for the entire tract and the general cropping plan for the field crops were left entirely to the Division of Agronomy and the cropping plan for the vegetable crops was left entirely to the Division of Horticulture. In the spring of 1925 a uniform application of potash at the rate of 400 lb. per acre and of phosphate at the rate of 200 lb. per acre was made over the entire tract. Figures 5 and 6 show the respective cropping plans and the kinds of crops raised in each of the two seasons. It will be noted that the field crops are replicated in all cases, and in some cases twice, while the vegetable crops are carried in single units only. This plan with the vegetable crops was due to lack of room for replications with any great number of crops, and it is, in a way, a handicap, as under this plan checks on yields can be secured only by comparing the yields of one season with those of another where weather conditions may be entirely different. It will be further noted that each crop, whether field or vegetable crop was carried in a strip of uniform width across all control plots.

## YIELDS

*Limits of Actual Control Zones.*—The yields for each of the two years are shown in Tables 1 and 2. The average actual depth of ground water table for each crop item was computed on the basis already described, according to the exact location of the item, and the classification into control zones was made on the basis of these actual depths rather than on that of the theoretical depth of control.

*Length of Growing Season.*—For all crops maturing by or before the latter part of August the growing season was assumed as four months long and for all crops not maturing until fall the growing season was assumed as six months long.

As is customary in research work of this character all yield items were rejected which, for unaccountable causes, differed unreasonably from the evident normal or average yields for the tract and season. The grass crops appearing in Fig. 6 were not planted until June 1926, hence, there will be no yields to report on these before 1927.

Tables 1<sup>1</sup> and 2<sup>2</sup> are largely self-explanatory but the following comments are pertinent:

<sup>1</sup> Yields furnished by F. W. McGinnis, Assistant Agronomist, Minnesota.

<sup>2</sup> Yields furnished by H. P. Traub, Assistant Horticulturist, Minnesota.

*Field Crops.*—In both seasons the general tendency seems to have been for the best yields to appear where the ground water table was held at the average intermediate levels of from 2 to 3½ feet below the surface but with certain marked modifications as follows:

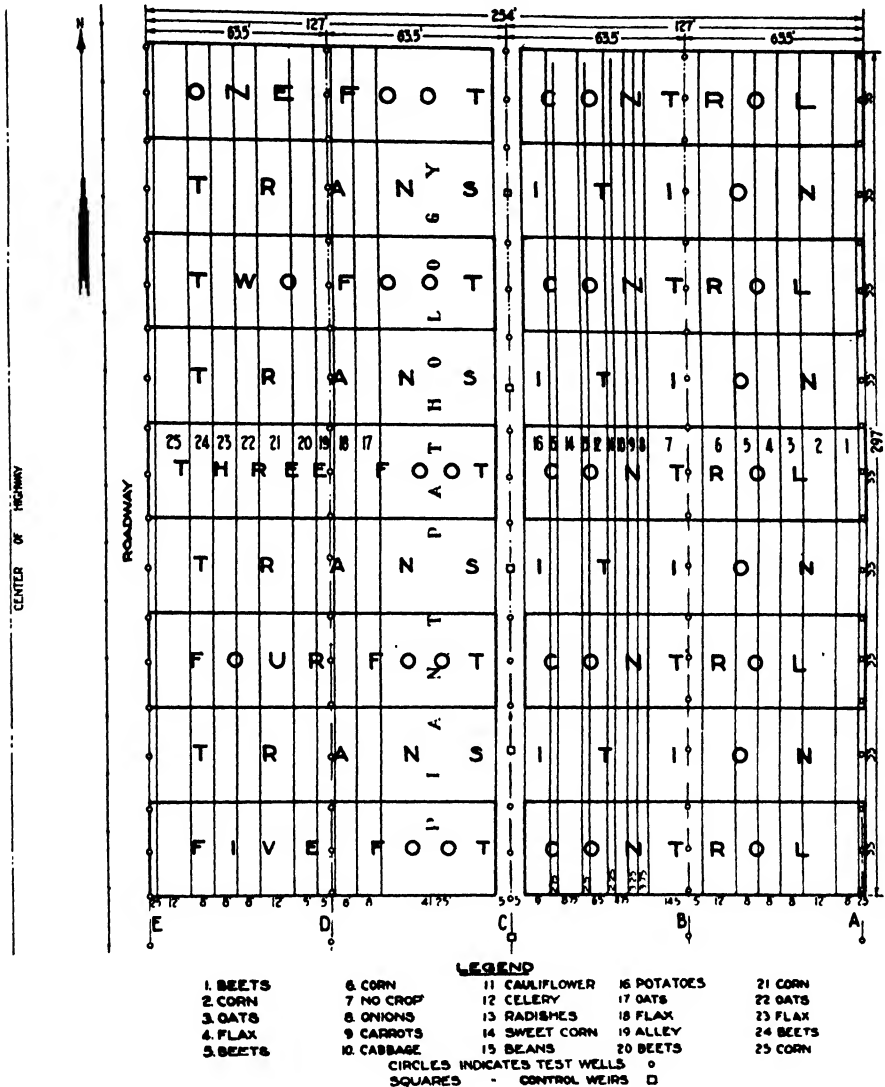


FIGURE 5.—Cropping plan for water-controlled peat plots for 1925, also showing location of test wells

1. Corn stover and sugar beets kept up their average yields through the deeper levels.
2. Straw and hay crops held fairly uniform in yields throughout *all* levels.



*Vegetable Crops.*<sup>1</sup>—In both seasons the general tendency seems to have been for the best yields to appear where the ground water table was held

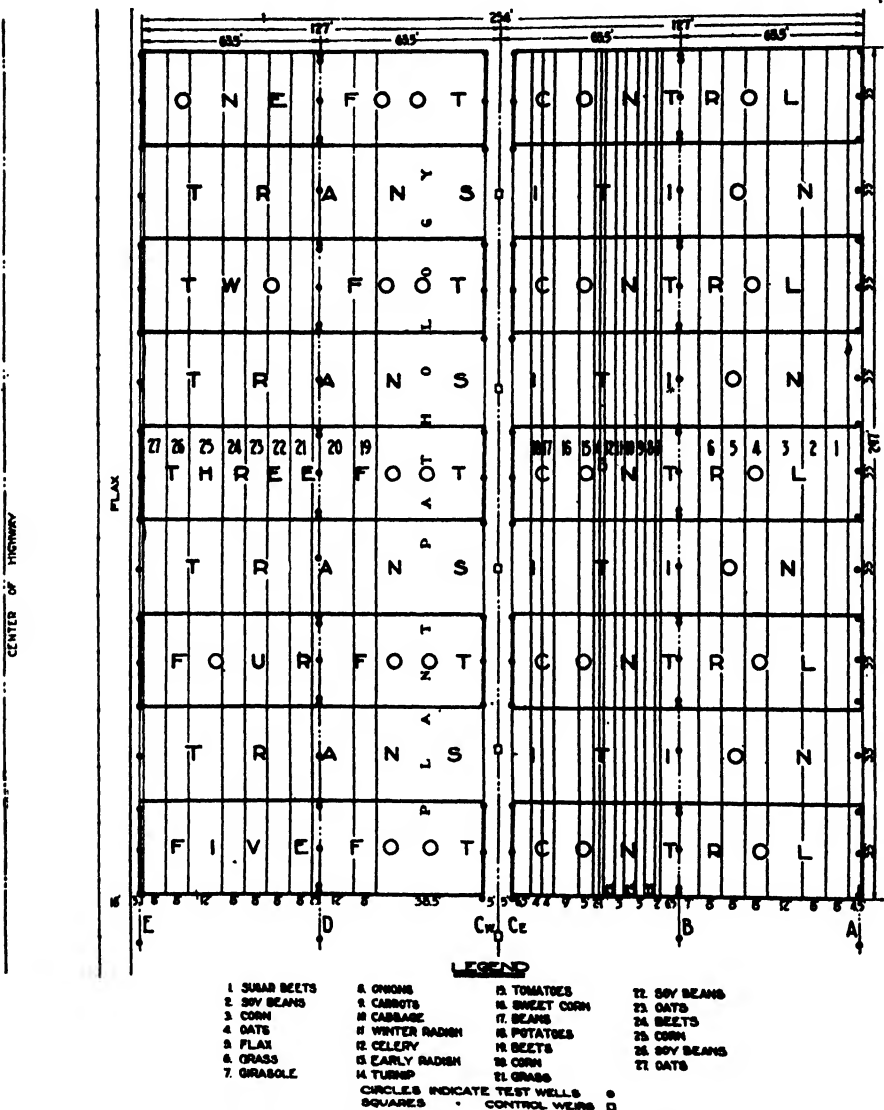


FIGURE 6.—Cropping plan for water-controlled peat plots for 1926, also showing location of peat wells

at the average intermediate levels of from 2 to 3½ feet below the surface but with certain rather marked exceptions as follows:

<sup>1</sup> H. P. Traub, Minnesota Journal Series Paper No. 759, entitled "The Effect of Height of Ground Water Table on the Development of Truck Crops on Peat Land." Proceedings of American Society for Horticultural Science, Nashville, 1927.

1. Celery did best with the ground water table 2 to 2½ feet below the surface.
2. Turnips did best with the ground water table 3 to 4½ feet below the surface.
3. Onions did best with the ground water table 4 to 4½ feet below the surface.

### COMPARISON OF YIELDS WITH APPROXIMATE STANDARD YIELDS

As a basis for grading the yields shown in Tables 1 and 2 the following approximate average yields are offered as a standard:

#### *Field Crops.—*

Ear corn, 42 bushels per acre  
Corn stover, 3 tons per acre  
Sugar beets, 10 tons per acre  
Flax grain, 10 to 11 bushels per acre  
Flax straw, ¾ ton per acre  
Oats, grain 34 bushels per acre  
Oat straw, 1½ tons per acre  
Soybean hay, 1¾ tons per acre

#### *Vegetable Crops.—*

Potatoes (on peat), 250 to 300 bushels per acre  
Beans (dry), 12 to 15 bushels per acre  
Sweet corn (green), 3 to 5 tons per acre  
Tomatoes, 12 to 15 tons per acre  
Turnips, no data  
Early radish, no data  
Winter radish, no data  
Celery, no data on weight  
Cauliflower, 5 tons per acre  
Cabbage, 18 to 20 tons per acre  
Carrots, 8 to 12 tons per acre  
Onions, 350 to 400 bushels per acre  
Girasole, 16 to 18 tons per acre

Comparisons, in general terms, of yields on this project, with the foregoing averages are shown in Table 3.

### SIMILARITIES AND DISPARITIES BETWEEN 1925 AND 1926 YIELDS

A study of the yields shown in Tables 1 and 2 raises the question in the case of most of the crops shown as to why there are such great disparities in yield in the two seasons, for any given crop, under apparently similar local conditions. If, however, we examine each case closely, making a

TABLE 3.—*Quality of crop, and comparison of yields with accepted average yields*

Kind of crop	Quality of best yield	Comparison of yield with accepted average yield	Approx. range of av. actual depth of water table, in ft. where best yields occur	Notes on quality at other depths of water table
Field crops				
Ear corn	Excellent	1½ to 2 times average	3-3½	Uneven on higher levels; uniform but smaller on lower levels
Corn stover	Low grade	Rather light	2-4½	Very uneven on higher levels
Sugar beets	Best on lower levels, rough on higher	Over double accepted average	2½-4½	Tendency to hollowness and rot on high levels
Flax grain	Good	Rather lower than average	3-3½ definitely	
Flax straw	Do	About 3 times accepted average, fairly uniform throughout all levels	2-3½	
Oats	Fair to rather poor	Less than ¾ accepted average		
Oat straw	Good	Noticeably above accepted average, fairly uniform throughout all levels		
Soybean hay	Do	Noticeably above accepted average on light to medium levels; good as average on deeper levels		
Vegetable crops				
Potatoes	Very good except on highest levels	Slightly better than average	2-3	Rough on higher levels, progressively smaller on lower levels
Beans	Good	Only about ½ average	3	
Sweet corn	Uniformly good on all levels at table stage	Average	2-4	
Tomatoes	Good	Do	2-3	Plants did not thrive on high levels
Turnips	Good on medium to lower levels	No basis of comparison	3-4	Fruit small on deeper levels
Early radish	Do	Do	2½-3½	Small on higher levels
Winter radish	Do	Do	3-4	Poor quality and rough on higher levels
Celery	Crop all good	Do	2-2½	Small on higher levels
Cauliflower	Fair	Do	2-3	Progressive decrease in size on lower levels
Cabbage	Good	Well up to average	2-3½	Fair yield throughout
Carrots	Good on all but high levels	Rather above average	2-3	Rough and poor development on upper levels
Onions	Good in 1925, poor in 1926	2 to 3 times average in 1925	4-4½	Yield drops off noticeably on lower levels
		Heavy but not marketable in 1926	2-4	Bulbs small on high levels and mostly scallions on lower levels
Girasole	Good	Comparable figures not obtained	2-3	Height of plants fell off progressively below 3-foot level

comparison of the conditions in the two seasons, that affect plant growth, such as, the fluctuation of the ground water levels owing to rainfall and the slow movement of the rain water through the peat soil, and owing to variable water requirements of the plant at different periods of its development, the variation in temperature, and the occurrence of frost or the lack of it, we may find good and sufficient reasons for existing variations in yield, and, at the same time, we may also find a way to modify some of the attendant conditions to such an extent as to help to stabilize the yields.

### WATER TABLE FLUCTUATIONS AND THEIR EFFECTS

From Table 3 it will be noted that the great majority of the crops under consideration yield best with the ground water table at intermediate depths below the surface, and it will be further noted that the yields for the same crops were nearly all lighter in 1926 than in 1925. A study of the ground water curves in Fig. 5 and 6 and of the rainfall records for the two years in Fig. 7 reveals the following interesting facts:

1. The average ground water level was noticeably higher, with reference to the actually existing surface each year, in 1926 than it was in 1925.
2. In 1926 rains occurred with greater frequency than they did in 1925 and the total rainfall, during the growing season, was much greater in 1926 than it was in 1925.
3. Excessive rainfall was much more noticeable during August, 1926, than during August, 1925, and August is a month in which many crops are maturing more rapidly than in other months, and are not, at this stage, in need of such large quantities of moisture as they are earlier in the season when the most rapid development of the plant is going on.

These facts may quite largely account for the lighter yields in 1926 with such crops as sweet corn, flax, oats, potatoes, beans, and onions.

### TEMPERATURE VARIATIONS AND FROST; THEIR EFFECTS

*Records Available.*—Some temperature records of the soil at different depths, and of the moving soil water were secured in 1925 but these were so meager that the record can be of little value in this discussion beyond corroborating, in a small way, the 1926 record of the tendency of the soil and water temperatures on the different controls, for the 1926 record, while by no means continuous and complete, was much more extensive than the 1925 record. The entire record of temperatures as secured in 1926 is entirely too voluminous to include in this paper but the monthly average temperatures and the maximum and minimum temperatures for each month, together with the dates on which they occurred, on the

different water control areas and the points and depths at which they were taken, are shown in Table 4.

Some of the temperature records most necessary to a thorough study of the problem before us, such, for example, as the minimum temperatures at and a short distance above the ground surface for each 24 hours, on the different water control areas, were not secured even in 1926, due, in part, to a lack of comprehension of just what temperature records would be most essential in study of the problem, and in part to a lack of the necessary apparatus and help.

*Relative Conductivity of Dry and Wet Peat.*—Nevertheless available temperature records, as epitomized in Table 4, when correlated with certain general facts in regard to water and peat soil temperature relations, well established by earlier investigations, reveal the following points of interest in this discussion.

Dry peat is a poor conductor of heat. The more it is compacted however, the better it will conduct heat. Water is a better conductor of heat than is dry peat. Moist peat will conduct heat better than will dry peat and the wetter the peat the better heat conductor it becomes. The conductivity of moist or wet peat lies somewhere between that of water and of dry peat.

*Tendencies of Temperature Variations on the Peat Bog.*—The general facts just stated serve, in large part, to justify the following specific facts brought out by a study of Table 4:

1. There is a progressive rise in temperatures of the peat soil at any given depth of 0.8 feet or greater, going from the higher ground water levels to the lower.
2. There is a progressive rise in temperatures of the peat soil at any given depth and for any given height of water table, from the beginning of the growing season forward to about the end of August, from which time forward through the fall a reversal of the progression occurs.
3. At a depth of 0.3 of a foot below the surface of the peat, which, it may probably be assumed with safety, lies within the surface layer compacted by the planting and tillage operations peculiar to peat land cropping, the tendencies of temperature variations stated under (1) just above, are reversed; but the tendencies stated under (2) hold here also.
4. The tendencies of temperature variations of the sub-irrigation water as taken in the center test well of each water control area, along the B and the D lines of test wells, follow the conditions stated under (3) just above.

*Coordinate Influence of Temperature and Rainfall.*—From a further correlative study of the rainfall record (fig. 7) and the ground water curves

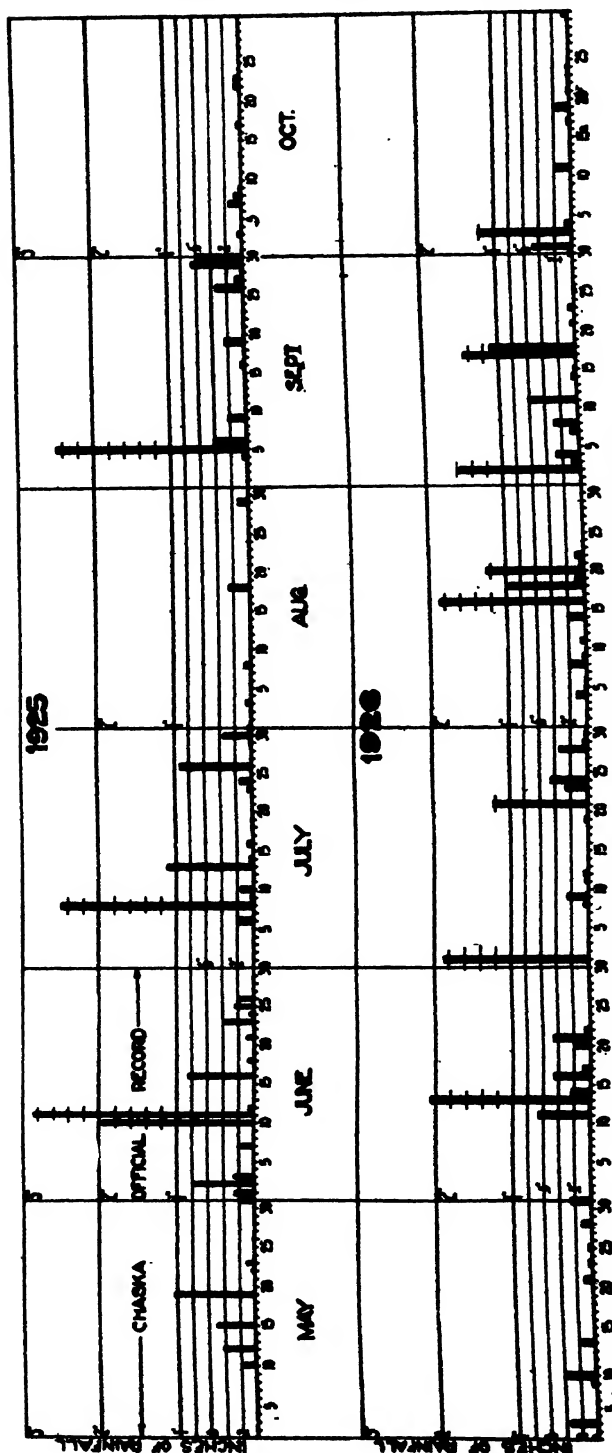


FIGURE 7.—Precipitation record at water-controlled peat plots

TABLE 4.—Temperature record, Fahrenheit scale, on water-controlled pent plots for 1926

Continuous thermograph record										Mercury thermometer record													
Foot notes	Location near well No.	Water table			Maximum			Minimum			Location near well No.	Water table			Soil 0.3 ft. below surface			Water in well					
		Theoretical depth in ft.	Av. actual depth in ft.	Depth of bulb in feet	Date	Time of day	Average temperature	Date	Time of day	Temperature		Theoretical depth in ft.	Av. actual depth in ft.	Temperature	Maximum	Average temperature	Minimum	Temperature	Maximum	Average temperature	Minimum	Date	
JUNE	D 2	1.0	1.2	2.0 49	6/21	8 P.M.	42	34	6/1	Mt.	B 2	1.0	2.6	66	6/10	62	59	6/14	54	6/14	51	46	6/3
	D 2	1.0	1.2	2.0 49	6/1	Mt.	42	34	6/1	Mt.	D 2	1.0	1.2	62	6/24	53½	53	6/3	52	6/14	45½	37	6/3
	B 10	3.0	3.0	0.8 58½	6/23	Mt.	53½	48	6/4	4 A.M.	B 6	2.0	2.5	60	6/10, 24, 28	59½	56	6/3, 7	49	6/28	44½	41	6/3
	B 10	3.0	3.0	0.8 58½	6/23	Mt.	53½	48	6/4	4 A.M.	B 10	3.0	3.0	57½	6/28	55½	54	6/3	47	6/21	43½	40	6/3
JULY	B 18	5.0	3.0	0.8 61	6/30	3 A.M.	52½	42½	6/4	4 P.M.	B 14	4.0	2.9	61	6/24	57½	54	6/3	46	6/28	44	41	6/3
	B 14	4.0	2.9	65	6/10	57½	50	6/3	47	6/28	D 14	4.0	2.9	65	6/28	57½	50	6/3	46	6/21	43	40	6/7
	B 18	5.0	3.0	64	6/28	57	53	6/3	42½	6/21	B 18	5.0	3.0	62	6/24, 28	59	57	6/3	42½	6/28	42½	40	6/3
	D 18	5.0	3.1	62	6/24, 28	59	57	6/3	42½	6/21	D 18	5.0	3.1	62	6/24, 28	59	57	6/3	42½	6/21	41	46	6/3, 7
(a)	D 2	1.0	1.0	2.0 59	7/28	3 P.M.	51½	49½	7/1	4 A.M.	B 2	1.0	1.4	72	7/29	67	65½	7/1	58	7/19, 22, 29	54	54	7/8, 12
	B 10	3.0	3.1	0.8 67	7/20	Mt.	63	58½	7/1	4 A.M.	D 2	1.0	1.0	72	7/8	65	61½	7/26	58	7/22	56	53	7/12
	D 10	3.0	3.1	2.0 62	7/19	Mt.	59½	58	7/31	M.	B 6	2.0	2.6	71½	7/8	66½	62	7/15	54	7/22	50	48	7/5
	D 10	3.0	3.1	2.8 54½	7/20	4 A.M.	53½	52½	7/31	2 P.M.	D 6	2.0	1.9	72	7/8	65	62	7/15	55	7/19, 26	52	50	7/1, 8, 12
(b)	B 18	5.0	3.4	0.8 69	7/21	4 A.M.	64	59	7/1	2 P.M.	B 10	3.0	3.1	73	7/8	66	63	7/15	52	7/19, 26, 29	45	47	7/1, 12
	B 14	4.0	3.2	68	7/8	61½	62	7/15	49	7/5, 19, 29	B 14	4.0	3.2	68	7/8	61½	62	7/15	49	7/5, 19, 29	48	46	7/1
	D 14	4.0	3.2	73	7/8	66½	61½	7/12	53	7/15	D 14	4.0	3.2	73	7/8	66½	61½	7/12	53	7/15	48½	45	7/1
	B 18	5.0	3.4	63½	7/15	62	60	7/12, 29	50	7/26	B 18	5.0	3.4	63½	7/15	62	60	7/12, 29	50	7/26	46½	44	7/1
(b)	D 2	1.0	0.9	2.0 59	8/2	8 P.M.	57	55	8/13	Mt.	B 2	1.0	1.4	71	8/23	68	66	8/16	62	8/19	59½	57	8/2
	B 10	3.0	2.9	0.8 66½	8/23	2 P.M.	64½	61½	8/14	8 A.M.	D 2	1.0	0.9	67	8/2, 19	63½	63½	8/26	64	8/16	57	53	8/2
	D 10	3.0	3.0	2.0 61	8/30	Mt.	59½	58	8/26	4 P.M.	B 6	2.0	2.5	70	8/23	66½	64	8/30	59	8/30	55	52	8/2, 12
	D 10	3.0	3.0	2.8 56½	8/23	Mt.	54½	53	8/13	4 P.M.	D 6	2.0	1.8	68	8/9	65	63	8/26, 30	62	8/30	55	52	8/2, 12
(c)	L 18	5.0	3.3	0.8 69½	8/23	Mt.	66	62½	8/14	M.	B 10	3.0	2.9	67	8/23	66	64	8/30	57	8/23	54½	50	8/2, 9
	B 18	5.0	3.3	2.8 60	8/18	Mt.	57	55	8/28	M.	D 10	3.0	3.0	68	8/2	65½	63½	8/26	56	8/23, 30	53½	50	8/5
	D 18	5.0	3.4	2.0 64	8/17	Mt.	62	59	8/29	Mt.	B 14	4.0	3.1	67	8/23	65	63	8/30	57	8/23	53	50½	8/2, 5
	D 18	5.0	3.4	5.0 55½	8/28	Mt.	55	53½	8/21	M.	D 14	4.0	3.0	69	8/23	65½	62	8/30	58	8/19	53	50	8/2, 5
(c)	D 18	5.0	3.4	2.0 64	8/17	Mt.	62	59	8/29	Mt.	B 18	5.0	3.3	67	8/19, 23	65	63	8/5	55	8/19	52	48	8/2
	D 18	5.0	3.4	5.0 55½	8/28	Mt.	55	53½	8/21	M.	D 18	5.0	3.4	68	8/2	66	64	8/30	58	8/19	55	52	8/12

TABLE 4 (Continued).—Temperature record, Fahrenheit scale, on water-controlled peat plots for 1936

		Continuous thermometer record										Mercury thermometer record												
Month	Foot notes	Water table				Maximum				Minimum		Location near well No.	Water table		Soil 0.3 ft. below surface				Water in well					
		Theoretical depth in ft.	Average depth in ft.	Depth of bulb in feet	Temperature	Date	Time of day	Average temperature	Temperature	Date	Time of day		Theoretical depth in ft.	Average depth in ft.	Temperature	Date	Time of day	Maximum	Average temperature	Temperature	Date	Time of day		
SEPTEMBER	B 10	3.0	2.4	0.8-66	9/1	M.	61	52	9/29	4 P.M.	9/29	B 2	1.0	1.3	62	9/6, 9	60	54	9/27, 30	61	9/2	59	54	9/30
	D 10	3.0	2.6	2.0-61	9/2	4 A.M.	59 1/2	56	9/29	8 P.M.	9/29	D 2	1.0	0.9	62	9/6, 9	58 1/2	51	9/27, 30	61	9/2	58	53 1/2	9/30
	D 10	3.0	2.6	2.8-57	9/5	Mt.	53 1/2	54	9/19	M.	9/19	B 6	2.0	2.1	67 1/2	9/2	59 1/2	57 1/2	9/13	60	9/6	57	53	9/30
	D 10	3.0	2.6	2.8-57	9/5	Mt.	53 1/2	54	9/19	M.	9/19	B 10	3.0	2.4	63	9/2	58 1/2	53 1/2	9/27	58	9/6, 20, 23	56	54 1/2	9/2
(e)	B 18	5.0	2.9	0.8-63 1/2	9/1	4 A.M.	63	53 1/2	9/29	6 P.M.	9/29	B 14	4.0	2.7	60	9/6, 9, 16, 23	58 1/2	53	9/27	59	9/23	56	54	9/27
	B 18	5.0	2.9	2.8-59	9/6	10 P.M.	57	54 1/2	9/1	10 A.M.	9/1	D 14	4.0	2.6	67	9/2	59 1/2	53	9/27	59 1/2	9/23	56	53 1/2	9/2
	D 18	5.0	3.0	2.0-61 1/2	9/14	2 P.M.	60	56 1/2	9/29	10 P.M.	9/29	B 18	5.0	2.9	66	9/2	59 1/2	53	9/27	59	9/23	55	53	9/2
	D 18	5.0	3.0	5.0-57	9/28	4 A.M.	55 1/2	54 1/2	9/13	2 P.M.	9/13	D 18	5.0	3.0	66 1/2	9/2	58 1/2	53	9/27, 30	58	9/2, 23	56	53	9/30
OCTOBER	B 10	2.0	2.4	0.8-56 1/2	10/4	M.	52	48	10/20	Mt.	10/20	B 2	1.0	1.3	60	10/4	54 1/2	54	10/11	56	10/7	55	54	10/11
	D 10	2.0	2.4	2.0-56 1/2	10/2	2 A.M.	55	51 1/2	10/9	Mt.	10/9	D 2	1.0	0.9	53	10/4	53	53	10/7, 11	53 1/2	10/7	53	53	10/7, 11
	D 10	2.0	2.4	2.8-54 1/2	10/4	10 A.M.	53 1/2	51	10/19	6 A.M.	10/19	B 6	2.0	2.2	58	10/7	55	55	10/7, 11	53 1/2	10/4	54	54	10/4
	D 10	2.0	2.4	2.8-54 1/2	10/4	10 A.M.	53 1/2	51	10/19	6 A.M.	10/19	D 6	2.0	1.6	54	10/7	53 1/2	53	10/11	56	10/7	54	54	10/11
(f)	B 18	5.0	2.7	3.8-59 1/2	10/4	M.	54	50 1/2	10/21	6 A.M.	10/21	B 14	4.0	2.7	58 1/2	10/4	54 1/2	53	10/11	56	10/7	54	53 1/2	10/11
	D 18	5.0	2.7	2.8-55	10/6	10 P.M.	52 1/2	51	10/21	4 A.M.	10/21	D 14	4.0	2.5	54	10/4	54	54	10/11	54	10/7	53 1/2	53	10/11
	D 18	5.0	2.9	2.0-56	10/1	4 A.M.	53 1/2	50 1/2	10/20	4 P.M.	10/20	B 18	5.0	2.7	55 1/2	10/4	53 1/2	52	10/11	56	10/4	54	53	10/11
	D 18	5.0	2.9	5.0-56	10/6	Mt.	54 1/2	54	10/15	4 P.M.	10/15	D 18	5.0	2.9	57	10/7	55	53	10/11	55	10/4	54	52	10/11

Thermograph record is for last 13 days of month only.

Do first half  
Do last 14 days  
Do Do half  
Do first 20 days of  
Do first 3 weeks of  
e Mt. stands for midnight.

NOTE.—Mercury thermometer readings were always taken as near the middle of the day as possible. These readings extend only through October 11th.



(figs. 5 and 6) it appears that the maximum height of the ground water curve resulting from a given rain, occurs at an appreciable interval of time *after* the rainfall and that this interval increases progressively with the normal depth of the ground water table. That is, following a rain and for some time after, owing to the slow downward movement of the rain-water in the peat soil, there is a zone of relatively dry peat soil lying between the surface layer, saturated by the rain, and the zone of moist or wet peat lying adjacent to and extending indefinitely below the ground water table. This relatively dry zone acts as an insulating layer between the two moist zones, checking any tendency to equalization of temperatures between the upper and the lower moist layers, and this insulating effect increases progressively with the depth of the ground water table below the bog surface. This general condition tends to exist at all times on the cultivated bog, owing to the packed condition of the surface of the peat, but it will be readily seen that it is greatly enhanced by rainfall.

*The Frost Menace.*—During the night the cooler air from the upper atmosphere is driven down over the bog by the warmer currents rising from the mineral soil adjacent to the peat bog as the mineral soil gives up its heat to the atmosphere much more rapidly than does peat soil under similar local weather conditions. The natural results of the facts and conditions above discussed relative to temperature and moisture variations in peat soil are what we might expect and are exactly what happen on the surface of a peat bog under cultivation. That is, progressively lower temperatures tend to occur, during the night time in the zones of the atmosphere lying next to the bog surface as we go from the areas of higher ground water level to those of lower ground water level, and such lower atmospheric temperatures also tend to occur more often and with increased intensity during periods of frequent rainfall than in drier periods. For the surface compact or moist layer of peat soil gives up its heat to cooler air passing over it much more rapidly than it can receive more heat through the looser or relatively drier insulating zone from the much warmer moist peat or irrigation water below. The bog surface, therefore, does not always continue to warm the air adjacent to it throughout the entire course of a cool night and this condition often results in untimely frosts on parts of the peat bog where the water table lies well below the surface even when the parts of the bog with the ground water level near the surface and the adjacent areas of mineral soil are not touched by frost at all.

*Disastrous Effects of Untimely Frosts.*—The frost menace thus brought about, often results in injury or destruction to crops on the peat bog at times when we would least expect it. This is what happened on the bog under consideration in this paper and for obvious reasons, in the light of the foregoing discussion, the areas where the ground water level was farthest below the surface suffered worst. During the season of 1925

the injury from frost on this bog was negligible, while in 1926 a frost on the night of June 24th seriously injured the beans and killed the corn and soybeans on the 3, 4, and 5 foot controls where, from the experience of the previous year, the best and biggest yields were to be expected, while the 1 and 2 foot controls, where the lower grades and yields are obtained, were not touched at all by the frost. Again, early in September 1926, a frost destroyed any chance of the soybean seed maturing and injured the corn stalks, delaying the maturing of the ears so that a frost later in the month fully killed the stalks ten days short of the full maturing of the ears and rendered the stover of no value. It will be noted that these experiences with frost in each case followed from 1 to 3 days after a rain or a rainy spell and although the data on them are none too exact they, nevertheless, give some pretty clear indication of why many of the 1926 yields are smaller than those of 1925.

The difference in yields of potatoes is, doubtless, owing largely to difference in variety. Triumphs, also, were planted in 1926 but the seed was poor and the plants were so unthrifty that they were rooted out.

#### TENTATIVE CONCLUSIONS AND RECOMMENDATIONS

The available data are still too meager and fragmentary to admit of the drawing of any dogmatic conclusions or of the making of any definite recommendations, but the following apparent facts and tendencies may be pointed out as a tentative guide to safe practice in cropping peat land with controlled ground water levels, until more exact knowledge, based on fuller investigation is available.

*General.*—In determining suitable depths for the control drain tile to secure given average depths of water table between drain lines, allowance must be made for:

1. Maximum height of the peak of the ground water curve, of from 0.5 of a foot to 1.0 feet above the normal water level over the tile lines according to the water permeability of the peat.
2. Settlement of the bog where it has not been previously drained, pastured or cropped, of from 0.5 of a foot to 1.0 foot according to the average depth of water table desired.

If it is possible to do such a thing as a protection against frost, which may occur at any time of the season even in midsummer, provision will be very desirable for a quick, light, surface flooding of the lower areas on cool nights, coupled with provision for equally quick surface removal of such flood in the early morning. \*

*For Field Crops.*—For general farming of peat soil for raising standard field crops, where rotation will be practiced both as a matter of convenience and good management, it will not usually be found feasible or profitable to attempt an elaborate ground water control system that will permit

of maintaining the ground water table at different depths on different fields or portions thereof. Hence, good practice will counsel the fixing upon an approximately uniform average depth of ground water table, for the entire field, which will most nearly approach the requirements of the majority of the crops to be raised, exclusive of the true grass crops. This depth is about  $3\frac{1}{2}$  feet. Hence, the control tile should be placed at this depth plus the allowance above mentioned for settlement of the bog—if it be a new, wild bog—and for arching of the ground water table, the total original depth at time of installation, in most cases, amounting to from  $4\frac{1}{2}$  to  $5\frac{1}{2}$  feet.

*For Vegetable Crops.*—The growing of truck crops on peat land is usually carried out on much smaller or more definite field units than those used for field crops in general farming. Hence, the control of the height of the ground water level to suit the individual requirements of different crops or groups of crops is a much more reasonable and economically sound proposition than it is in the case of general farming. In fact where small peat areas are used for the intensive growing of truck crops and where a reliable source of water supply is available, the installation of such a system of ground water control seems to the writer the wisest thing to do. In such a case the data available point to the following depths of control of the average ground water table as good practice.

	Feet
For tuber crops such as potatoes and girasole	$2\frac{1}{2}$
tomatoes and celery	$2\frac{1}{2}$
beans	3
sweet corn, cauliflower, cabbages and root crops such as	
turnips, early radishes, winter radishes, and carrots	$3\frac{1}{2}$
onions	4

In determining the depths for the control tile, allowances should be made the same as stated under *Field Crops* for the settlement of the bog and the arching of the ground water table.

### FURTHER NEEDED INVESTIGATION

Before justification can be had for drawing dogmatic conclusions and making recommendations accordingly in this problem, much more investigation is necessary. The following are among the most important things to be carried out:

1. A third season, at least, of securing cropping data with different controlled ground water levels; three more seasons would be better.
2. At least one check year of cropping without varying the water level on the different control plots but rather with the water table held low over the entire tract.

3. Three more seasons of keeping exact temperature records as follows:

(a)<sup>1</sup> Maximum and minimum daily temperatures of the air at the bog surface and one foot above it on at least the 1, 3, and 5 foot control levels.

(b) Daily maximum and minimum temperature readings within the seed bed zone, that is, not over 0.3 feet below the surface on at least the 1, 3, and 5 foot control levels.

(c)<sup>1</sup> Continuous thermographic records on the 1, 3, and 5 foot controls, at depths as follows:

one foot control, 0.8 and 2.0 feet

three foot control, 0.8, 2.0, and 4.0 feet

five foot control, 0.8, 2.0, 4.0, and 5.0 feet

(d) Semi-weekly temperatures by standard thermometer, of the irrigation water in the test wells in the center of each control plot on each side of the main tile.

4. Precipitation records throughout the year for the entire further period of investigation.

5. Extending the range of field crops to include grass crops and clover and possibly field peas, and of vegetable crops sufficiently in point of time to give at least three years check on all crops grown.

6. Taking necessary engineering steps to secure more exact control of the ground water levels at or near the theoretically established elevations on the 1, 4, and 5 foot levels.

It is the hope of the writer that the Minnesota Station will be able to cover practically all these phases of fuller investigation and some provision is already made therefor.

<sup>1</sup> H. P. Traub and C. E. Steinbauer, Minnesota Journal Series Paper No. 760, entitled "Summer Frost Prevention on Northern Peat Lands by Raising the Ground Water Table." Proceedings of American Society for Horticultural Science, Nashville, 1927.

# THE WORLD'S RESOURCES IN AGRICULTURAL POTASH

J. W. TURRENTINE

*United States Department of Agriculture, U. S. A.*

## INTRODUCTION

It is not hard to imagine what may have been the original use of potash fertilizer. The primitive agriculturalist, proceeding with but scant experience and without any scientific theory or knowledge, knew nothing of plant foods, but it was not difficult for him to observe that wood ashes when scattered casually from his crude fireplaces caused a different character, a more desirable type of plant growth. In this inadvertent use of wood ashes, he was obtaining and could observe not only the beneficial effects of potash as a plant food, but also that of the lime and likewise the results of the elimination of soil acidity by the alkaline carbonates present. The benefit was three-fold and for that reason was less mistakable.

The agricultural use of potash as wood ashes is recorded in the early writings on agricultural subjects, but it was not until the completion of the classic researches of Liebig in 1860 that the underlying reasons for that use were appreciated and even then that knowledge would have been of little value, perhaps, had it not been for the fact that almost simultaneously there was discovered the great German potash deposits followed by their brilliant exploitation for the benefit of the agriculture of the world. The German industrialist was quick to grasp the potential importance as a commodity for world trade of the vast accumulation of potash which he found in the Stassfurt region and which, when first discovered, he regarded as only a useless and bothersome material.

So closely did the discovery of great potash deposits follow upon the heels of the scientific discovery of the basic principle underlying the agricultural value of potash, that the agriculturalist found himself urged to buy potash almost before he actually knew that he needed potash as an article of plant food. It may be said that the desire to sell, on the part of the German industrialist, antedated the farmers' desire to buy potash. The farmers' education in the use and value of potash came about as the direct result of the producers' desire to sell. Thus, the German potash producer, through his extensive research, demonstration and propaganda, became the world's greatest teacher in the agricultural use of potash. In this he was ably assisted and abetted by the agricultural experimentalists the world over, until today potash is universally recognized as a mem-

ber of the reigning triumvirate of plant food elements—nitrogen, phosphorus and potassium—on which is based the great fertilizer industries of the world.

The value of potash as a fertilizer having been established and the agricultural world having been committed to its use as an essential of efficient crop growing, it was natural that there should arise a feeling of uneasiness at the fact that the world's visible supply was in the possession of one nation and of one group within that nation. It was evident what great powers for levying tribute on world agriculture that situation represented. There had been no great evidence of undue use of those powers. The monopoly in potash had been benign. So far, the benefits of educational propaganda and abundant supplies at moderate rates had vastly preponderated. It was not certain that their régime would be maintained and the temptation indefinitely resisted to reap the fortune that apparently was ripening for the harvest. It was deemed the better part of wisdom by the several nations depending on German potash to achieve at least a moderate degree of independence and to establish domestic sources of this agricultural essential.

This sentiment apparently was world-wide, for practically simultaneously many nations began to concern themselves with the establishment of potash industries of their own. As a consequence, where less than a decade ago, Germany held what appeared to be an almost impregnable monopoly of the world's potash business, there are today in operation potash industries in some ten different countries. The whole industrial world, it might almost be said, is busying itself with the task of increasing the world's supplies of agricultural potash. Can there be any doubt as to the outcome?

In the year 1926, the world production of potash salts was approximately 11 million tons, equivalent to  $1\frac{1}{2}$  millions tons of actual potash ( $K_2O$ ). Of this total, as shown in the following tabulation, Germany

*The World's potash production in 1926*

Country	Potash salts	Actual potash ( $K_2O$ )
Africa	1,200	600
Chile		
France	2,317,500	351,000
Germany	8,500,000	1,099,740
Italy	3,600	1,800
Japan		
Poland	207,700	31,800
Russia		
Spain	5,000?	1,000?
United States	46,320	23,366
Total	11,081,320	1,509,306

produced 66 per cent and France 23 per cent, the balance of 11 per cent being produced by several other nations. These various potash industries will be presented in some detail in order that a picture of the world's potash resources and present industries may be shown.

## GERMANY

The German Potash Syndicate as now organized and equipped, while producing at the rate of 9,000,000 tons of potash salts per annum, a production determined by present demand, has a production capacity, it is estimated, of 20,000,000 tons potash salts equivalent to 2,000,000 tons of actual potash ( $K_2O$ ).

In accordance with the potash agreement now in force between the German and French producers, Germany has exclusive rights to her home market and that of her colonies, protectorates and mandates and 70 per cent of the foreign market, the balance of 30 per cent being retained by the French, a factor tending to restrict the development of the German production to present output. With advances in prices under the present strategic price-fixing arrangement, the increase in transportation costs and the development of potash industries closer to the areas of potash application, it is probable that the great and efficiently developed German deposits, containing, it is estimated 2 billion tons of actual potash, will for many years from necessity be held largely in reserve. On the basis of these estimates and at the current rate of potash use, the German deposits alone are capable of supplying the world's potash requirements for 2,000 years. That future explorations will disclose additional deposits seem quite probable.

The following statistical outline presents the present performance of the German potash industry.

The year 1926 did not bring the prosperity to the German potash industry that was anticipated with the developments that were under way in 1925. Potash sales for the year about equaled those of 1913, the last normal pre-war year, but proceeds, according to estimates, fall short of those formerly obtaining, being only 14 marks per 100 kilos in 1926, as compared with 17 marks in 1913, or a total of 150 million marks net as contrasted with 190 million in the pre-war year.

Entering into this unfavorable situation are the facts that plans made for anticipated sales of 1.5 to 2 million tons pure potash were not realized; interest of 20 million marks on the 300 million mark loan had to be met, and the new and expensive mass-production units which it was confidently expected would reduce production costs, were never operated at full capacity. For example, the Merkers works, built to treat the output of the Kaiseroda Shafts in Thuringia, the largest plant in the world, with a daily capacity of 5,000 to 6,000 tons, raw salts, because of present allocation laws was not permitted to operate at a rate greater than 1,000 tons per day.

To meet the situation, German producers petitioned the Federal Potash Council for a price increase of 18 per cent, which was denied; but on December 23, were granted a price increase of 9.5 per cent.

The result of this increase as reflected in the inland price of various grades of potash for 1926, as contrasted with 1925 and 1913, is shown in the following table:

*Present inland prices, in gold marks per 100 kilos pure potash ( $K_2O$ ) effective December 23, 1926, as compared with those for 1925 and 1913*

	1913	April 16, 1925	Dec. 23, 1926	Per cent comparison preceding	Per cent comparison 1913
Carnallite, 9-12 per cent $K_2O$	8.50	7.56	9.67	Plus 27.9	Plus 13.8
Kainite, 12-15 per cent $K_2O$	10.00	8.97	10.83	Do 20.7	Do 8.3
20-22 per cent fertilizer	14.00	12.24	15.20	Do 24.2	Do 8.6
30-32 per cent fertilizer	14.50	15.64	17.95	Do 14.8	Do 23.8
49-42 per cent fertilizer	15.50	16.68	18.88	Do 13.2	Do 21.8
Potassium chloride—50-60 per cent $K_2O$	27.00	27.00	27.00		
Potassium chloride—over 50 per cent $K_2O$	29.00	29.00	29.00		
Potassium sulfate, over 48 per cent $K_2O$	35.00	31.25	31.25		Minus 10.6
Potash magnesia sulfate, 26 per cent $K_2O$	31.00	28.85	28.85		Do 6.9

In the following table are given the monthly sales of potash by the German Potash Syndicate for the year 1913, 1925 and 1926.

*Monthly sales of potash by the German Potash Syndicate  
(In metric tons  $K_2O$ )*

Month	1913	1925	1926
January	11,550	165,990	94,970
February	168,880	202,050	185,510
March	134,730	143,980	139,110
April	52,710	66,810	57,290
May	47,940	73,210	41,750
June	45,490	81,610	82,820
July	57,640	95,620	86,550
August	111,560	104,550	101,950
September	115,860	101,270	82,600
October	80,930	73,720	63,090
November	98,350	57,690	68,370
December	84,680	58,920	95,730
Total	1,005,320	1,255,420	1,099,740



At the close of the year 1926, there were 228 potash works, inclusive of the newly opened Baden mines, holding production quotas in the German Potash Syndicate; of these, 42 major works and 21 minor subsidiaries were in operation. Of the remaining 165 non-productive units, 47 were being held in reserve, while 118 had closed down voluntarily until 1953.

The following table describes the present organization of the German Potash Syndicate, the concerns or groups comprising it, the production quotas held, and the number of shafts now operated by each.

*Organization of the German Potash Syndicate*

Name of concern	Production quota	Quota-bearing shafts	Shafts now running
	per cent		
Potash Syndicate (total)	100	224	63
Wintershall	39.0	89	11
Salzdefurth-Aschersleben-Westeregeln	24.2	46	14
Burbach-Gumpel	17.7	36	16
Prussian State	5.9	11	7
Neutassfurt-Friedrichshall	4.5	10	3
Solvay	2.5	5	5
State of Anhalt	2.1	6	2
Sauer	2.1	5	3
Others	2.0	16	2

*Production Costs.*—Figures presented by the German Potash Syndicate to the Federal Potash Council representing potash production costs and supporting the syndicate's petition for an 18 per cent price increase are tabulated below.

*Production costs in gold marks per 100 kilos  $K_2O$  as contrasted with 1924 and 1913*

	1913	1924	1926
Wages and salaries	2.56	4.83	4.24
Coal	2.40	3.80	1.47
Materials	5.08	3.39	1.82
Amortization	3.26	3.26	3.26
Administration and operating costs			0.75
Interest			
Quotas			1.18
Total	13.30	15.28	12.72

These figures, it is contended by opposing interests, are too high, being the average for all works, and not for the larger and cheaper producers,

the figure 8.45 marks probably more accurately representing the true costs. To this should be added the foreign loan levy of 2.07 marks per 100 kilos (being 7 per cent on 300 million marks) and the Dawes Plan commitment (123 million marks) of 0.56 marks, raising the total amount to 11.08 marks per 100 kilos  $K_2O$ . With an average sales price of 12.79 marks, a profit of 1.68 or 15 per cent on the investment it is alleged is realized.

Total sales of German potash for the year 1913–26, inclusive, are tabulated below:

*Sales of German Potash*

*(In short tons of  $K_2O$ )*

Year	Total	Year	Total
	tons		tons
1913	1,221,406	1920	1,017,856
1914	994,387	1921	1,118,996
1915	747,754	1922	1,445,667
1916	972,373	1923	990,000
1917	1,104,709	1924	843,860
1918	1,101,830	1925	1,225,454
1919	893,201	1926	1,099,740

*Exports.*—Potash exported by the Syndicate in 1926 fell to 415,000 metric tons of pure potash ( $K_2O$ ) as contrasted with 453,000 tons in 1925 and 506,100 in 1913. Exports, for these years, of potash salts and other compounds produced as by-products of the potash industry are shown in the following tabulation.

*Exports by the German Potash Syndicate 1913, 1925 and 1926, in metric tons*

Item	1925	1926	
		Total	United States
Carnallite, 9–12 per cent $K_2O$	445	422.5	
Kainite, 12–17 per cent	317,028	267,092	112,222
Fertilizer salts, 18–42 per cent	828,553	695,769	216,110
Potassium sulfate	129,369	133,425	58,148
Potassium chloride	196,672	201,319	120,851
Potassium magnesium sulfate	40,233	49,257	12,152
Calcium chloride and magnesium chloride	46,991	52,533	14,073
Bitter salt (magnesium sulfate)	32,058	39,526	4,314
Potassium chlorate	15,877	19,510	6,541
Potassium carbonate	12,213	14,642	4,505
Potassium manganate and permanganate	413	851	
Potassium and other bromides		802	90

A new potash mine has been created at Buggingen, Baden, 20 miles distant from the Rhine. A shaft begun in 1911 has now at a depth of 2,600 feet reached high grade potash salts supposedly a continuation of the Alsatian deposits. A second shaft has been begun. Public funds to the amount of 8,540,000 marks have been appropriated to further this development.

## FRANCE

France is the second largest producer of potash by virtue of the fact that when Alsace by the treaty of Versailles was ceded to France, she thereby acquired the already developed Alsatian potash properties. These were formerly a part of the holdings of the German Potash Syndicate and had been developed by that group, although that development had been restricted to prevent undue competition with the more important and more influential properties centered in Stassfurt.

Following the Peace, the Alsatian mines with the exception of those owned by the Alsations (The Sainte Therese group) were put into operation as state properties. Under the French régime they have been operated at full capacity inasmuch as under the German-French agreement, 30 per cent of the foreign market has been allocated as the French quota, providing an outlet for the entire output of the Alsatian mines.

Estimates of potash reserves based on present surveys of the Alsatian deposits indicate a total of 300 million tons of actual potash. The deposits contain principally carnallite and sylvinite, averaging about 18 per cent  $K_2O$ . While these salts are somewhat richer than those constituting the main German deposits, they are lacking in sulfates, which fact is detrimental in restricting the output of a varied assortment of fertilizer salts and likewise of by-products. Also, they are less advantageously situated with respect to accessibility to water transportation facilities. The present situation within the French potash industry is presented in the following statistical statement.

Production of the French potash mines under State control for the year 1926 was 1,672,500 tons salts as contrasted with 1,440,800 tons in 1925, an increase of 16 per cent; and by the Sainte Therese group, 645,000 tons, as contrasted with 485,000 in 1925, an increase of 32 per cent. The total for the two production groups was 2,317,500.

The production of potassium chloride by the two was respectively 237,000 tons and 125,000 tons, or a total of 362,000 tons.

Potash sales for the year amounted to 351,000 tons  $K_2O$ , an increase of 29,000 tons over that of the preceding year. Of this, 146,000 tons entered the domestic market and 205,000 tons were exported. Of exports, 50 per cent was shipped to America, 19 per cent to Belgium and 17 per cent to the British Isles.

Production of potash salts by the French mines for 1913 and 1919-26 inclusive is presented in the subjoined table:

*Production of French Mines, 1913 and 1919 to 1926, in metric tons potash salts*

Year	Société de Kali Sainte-Therese	Sequestered mines	Total
	metric tons	metric tons	metric tons
1913			350,000
1919	89,000	500,000	589,000
1920	156,000	1,066,000	1,222,000
1921	145,000	759,000	904,000
1922	382,000	2,945,000	3,327,000
1923	446,000	1,132,000	1,578,000
1924		1,230,000	1,230,000
1925	485,000	1,440,800	1,925,800
1926	645,000	1,672,500	2,317,500

Exports for the years 1925 to 1926 in metric tons of the various potash salts are shown in the following table:

*Potash salts exported from France in 1925 and 1926*

	1925	1926
	metric tons	metric tons
Carnallite, sylvinites	679,577	629,778
Potassium chloride	43,014	46,153
Sulfate of potash-magnesia	31,112	24,866
Potassium sulfate	949	1,695

Shipments of potash salts from the Alsatian mines to the United States for the year 1921 to 1926, inclusive, were as follows:

*Potash salts shipped to the United States from French mines, 1921 to 1926, inclusive*

Year	Tons
1921	86,870
1922	272,929
1923	361,621
1924	341,776
1925	333,925
1926	318,579 <sup>1</sup>

During the year, a new refining plant was put into operation at the Marie-Louise mine, near Staffelfelden. This plant for the production of potassium chloride, has a reported capacity of 120,000 tons of that salt per annum, a production which it is anticipated will be reached in two years.

<sup>1</sup> First eleven months.

The French mines and refining plants are being operated at full capacity, since in view of the fact that the modernized German industry will have a production capacity ten times that of the French, dissatisfaction is being expressed at the high ratio allowed the French producers under the German-French potash agreement. Accordingly, increased production is striven for and increased markets both at home and in America. Propaganda is freely used to the latter end, and with the same objective efforts are made to maintain prices at the present level. This, it is argued in Europe, should be considered of especial importance as operating to discourage the development of the American potash industries.

*Prices.*—On November 15, a domestic price increase of 5 per cent for all grades of potash was put into effect by the French potash selling agent. Present prices per 100 kilos are, therefore: Sylvinite, 10.95 francs; rich sylvinite; 17.50 francs; Potassium chloride, 72 francs; potassium sulfate, 98.70 francs.

## POLAND

There are two regions in Poland where the occurrence of potash salts has been well established. These are the western section comprising a large portion of the provinces of Posen and Lodz, and the southeastern section in the Carpathian foothills including parts of the Galician provinces of Lawes and Stanislawow. The latter deposits are of greater and more immediate importance as they are more fully exploited and are better known. They extend for a length of about 50 miles between Kalusz and Stebnik, and are  $1\frac{1}{2}$  to  $2\frac{1}{2}$  miles in width. In this region the beds, 4 to 60 feet in thickness, are found at depths of 200 to 750 feet.

Mining began as early as 1862, but under the restrictions imposed by the Austrian Government was but slightly developed prior to 1900. Following the Peace and with the restoration of Galicia to Poland, the properties were placed under development with the production in 1920 of about 6,800 tons of potash salts, accompanied by explorations, the discovery of new deposits and the organization of new operating companies. As the result of these explorations, present estimates place the Polish potash reserves at 18–20 million tons of potash salts, which on the basis of an average content of 25 per cent  $K_2O$ , is equivalent to 4 to 5 million tons of actual potash. Areas known to be underlain with potash salts but so far unexplored are expected to raise this total considerably.

The two properties producing the bulk of the present output are located at Kalusz and Stebnik. Only crude salts are at present being marketed, although refining plants are now approaching completion which will provide for the manufacture of a very considerable tonnage of refined or high-grade salts. Nationalistic aspirations are encouraging the exploitation of the Polish potash deposits as a part of the nation's program for developing and utilizing natural resources.

Production of Polish potash for the period 1920 to 1926 inclusive, as well as for 1913, the last year under Austrian control, is shown in the following tabulation.

*Output of Polish potash mines, 1913 and 1920 to 1926, inclusive, in metric tons*

Year	Kainite	Sylvinite	Total salts
1913	2,344		2,344
1920			6,789
1921	182	15,329	15,511
1922	2,520	43,563	46,083
1923	22,128	39,375	61,503
1924	23,545	57,875	81,420
1925	62,823	115,980	178,803
1926	79,166	128,523	207,689

## RUSSIA

Announcement has been made of the discovery and preliminary surveys of what authentically appear to be very important deposits of potash salts in the Salikamsk district of Russia. The area so far explored is only 100 square kilometers, in which 9 borings have been made with core drills. The deposit is apparently centered under an area of approximately 400 square kilometers, situated between Salikamsk and Bereznikov.

The deposit is described as consisting of strata of "overlying salts" 3 to 47 meters thick which gradually merge with carnallite or secondary sylvinite, which in turn overlies carnallite strata of some 80 meters in thickness. At lower levels, sylvinite is again encountered and finally rock salt.

On the basis of the first seven borings, the following summary of findings is given:

**Carnallite Zone**—depth 103 to 185 meters; total thickness, 82 meters; total workable strata, 43 meters; average content, 18.5 per cent KCl.

**Sylvinite Zone**—depth 189 to 220 meters; total thickness, 31 meters, total workable strata, 16 meters; average content, 25 per cent KCl.

In the area surveyed by the first five complete borings, it is calculated that underlying each square kilometer there are 11,467,000 tons of actual potash ( $K_2O$ ) or a total of 68,000,000 tons for the 6 square kilometers involved. More recent reports are to the effect that at present a total of 9 borings have been completed extending the area surveyed to 100 square kilometers. In the absence of definite information on that point, it is doubtful if one is justified in assuming the continuity of the deposit over this area in the same thickness and concentration as that more intensively examined by the earlier explorations. If one made such an assumption, however, the total of 11 billion tons actual potash is arrived at

as the estimated potash content of the portion of this deposit already surveyed.

The Superior Economic Council of Russia has organized the Union Potash Trust to exploit this deposit and construction has begun on the first works situated south of Solikamsk to be completed in 1928, while pioneering is being continued near Bereznikov. A railroad is to be constructed connecting Solikamsk with Ussolye.

The apparent enormity of these deposits does not neutralize the unfavorable aspect of their isolation. For agricultural Russia they will eventually be a most important asset, but for the rest of the world they will be of little importance.

## SPAIN

The Spanish potash deposits which occur in Catalonia, were discovered in 1912 and accordingly are still imperfectly explored and developed. Only about 25 shafts have so far been sunk, ranging in depth from 305 to 1187 meters. The potash strata so far penetrated vary in apparent thickness from 35 to 205 meters and occur at depths of from 200 to 800 meters.

These deposits are made up principally of the potash minerals, carnallite and sylvinite, associated with rock salt. They underlie a region 75 miles long by 18 wide, and are estimated to contain 2 billion tons of salts equivalent to 268 million tons of actual potash ( $K_2O$ ). Present developments will provide for a daily capacity of 10,000 tons crude salts, the objective being the preëmption of the home market with the exportation of a limited surplus. The Spanish government has enacted laws and regulations imposing an import tariff on foreign potash as a protection for the domestic industry, and limiting output and exports and controlling both domestic and export prices.

## UNITED STATES

While for many years the United States has been one of the great potash using countries, prior to 1911 it gave little thought to its own potash potentialities. In that year, surveys were inaugurated to determine whether or not there existed within its own boundaries deposits of raw materials from which could be manufactured in part at least the potash for which at that time the nation was entirely dependent on Germany. It was the realization of this state of utter dependence that first inspired the desire that we establish our own industry if not to supply our own requirements in this agricultural essential, at least to afford some defense against unrestricted exploitation by a foreign monopoly.

Surveys conducted by the Federal Government embraced American salines, both the subterranean deposits of the states of New York, Ohio, Michigan, Kansas and Louisiana and the surface deposits of the states of

Nebraska, Utah, Nevada, Oregon and California; the kelps of the entire Pacific littoral; the alunites of Utah, the leucites of Wyoming, the potash shales of Georgia, the feldspars of various states—all natural potash carriers; and cement dust, blast furnace dust, distillery waste, wool-scouring and Steffen's waste from beet sugar mills, industrial wastes—all of which were subjected to varying degrees of chemical investigation to determine methods of extracting potash therefrom. Practically all of these raw materials made contributions to the nation's supplies of potash during the war years.

Following these surveys came the European war with the complete cessation of potash importations. America was thrown upon her own resources and happily had at hand the newly acquired information gotten in these surveys, so that it was possible to proceed with intelligence and dispatch in the establishment of potash industries.

During this period, the American sources of potash which were placed under development were, principally, Nebraska Lakes brine, Searles Lake brine, Salduro Marsh brine, Salt Lake brine, kelp, cement dust, blast furnace dust, Steffen's waste, distillery waste, alunite, leucite, greensand, Georgia shales and wood ashes.

The following table gives the potash produced from the various sources during the war years, together with the price obtained:

*Domestic production of potash in the United States, 1915 to 1922, inclusive*

Year	Crude potash	Available content of potash ( $K_2O$ )	Value
	short tons	short tons	
1915	4,374	1,090	\$342,000
1916	35,739	9,720	4,242,730
1917	126,961	32,573	13,908,577
1918	207,686	54,803	15,839,618
1919	116,634	32,474	11,271,269
1920	166,834	48,077	7,463,026*
1921	25,485	10,171	447,859*
1922	25,176	11,714	463,512*

\* Value of amount sold.

It will be observed that domestic production reached a maximum of about 55,000 tons  $K_2O$  in 1918, and in this connection it should be stated that at that time additional and enlarged plants were about to be put into commission which would have doubled this amount. So that it may be said with fair accuracy that we had developed a producing capacity of approximately 100,000 tons of  $K_2O$ .

With the reappearance of German potash on the American market, American potash industries for the most part suspended operations.



However, it should be noticed that certain industries have been able to withstand this period of exceedingly severe economic competition and are now producing potash of an excellent grade and of a tonnage which though small is highly significant.

As the result of these pre-war surveys, war-time exploitation of the various potash sources and post-war experiences under severe competitive conditions, we have acquired valuable information as to America's potash resources and the various economic and other elements which constitute America's potash problem.

The American potash industry has succeeded in maintaining its position in the face of the increasingly formidable European competition. While there has been no increase in production, the foundation has been extended for an enlarged industry both by the increase in production capacity of present plants and the establishment of new ones. These new developments while moderate, are conservative and well considered and offer the promise of a gradual though certain growth of the American industry on the broad foundation of potash as a product of various raw materials or as a side-product of a diversified manufacture.

Total production in 1926 amounted to 46,324 short tons of potash salts, equivalent to 23,366 tons of actual potash ( $K_2O$ ). Sales by producers amounted to 51,369 tons of salts containing 25,060 tons actual potash and valued at \$1,083,064 f.o.b. plants. About 26,000 tons of salts equivalent to 9,000 tons  $K_2O$ , remained in stock at the end of the year.

On the facing page is a record of American potash production for the period 1917 to 1926, inclusive.

The potash now produced is derived principally from desert lake brines and in part as a by-product of the alcohol, the cement and the blast-furnace industries. It is cheap potash because it is produced with by-products, to share its cost of manufacture. That is the basis of its economic strength.

The logical development of the industry as now constituted would yield our normal potash supplies. From the cement and blast-furnace industries alone, the potash now thrown away annually (175,000 tons in quantity) approaches our importations.

Among our undeveloped potash deposits—potash minerals—the green-sand marls of New Jersey alone contain enough potash within reach of the steam shovel to supply our requirements, at the present rate of consumption, for 1,000 years. Additional quantities in New Jersey, Delaware, Maryland and Virginia increase this amount many fold. These deposits constitute our greatest present-known resource in potash and possess the great advantage that they are practically surface deposits, can be mined by the steam shovel, require no blasting or grinding and are ideally situated with respect to industrial centers, transportation facilities and the fertilizer market. The Bureau of Soils has already developed a chemical

process whereby the potash and a number of by-products of value and importance may be obtained from greensand, representing practically 100 per cent utilization of the raw material. Supported by the side-products, the potash from this source would be able to meet the competition of that from any other source. This process is now in commercial operation.

*Potash produced and sold in the United States, 1917 to 1926, inclusive*  
(In short tons)

Year	Number of plants		Production			Sales*	
	Total	Exclu- sive of produ- cers of wood- ash potash	Crude potash	Available content of $K_2O$	Crude potash	Available content of $K_2O$	Value f.o.b. plant
1917	95	46	126,961	32,573	126,961	32,573	\$13,980,577
1918	128	77	207,686	54,803	140,343	38,580	15,839,618
1919	102	67	116,634	32,474	166,063	45,728	11,271,269
1920	66	49	166,834	48,077	139,963	41,444	7,463,026
1921	20	19	25,485	10,171	10,337	4,408	447,859
1922	12	12	25,176	11,714	22,028	11,313	463,512
1923	12	12	39,029	20,215	35,164	19,281	784,671
1924	11	11	43,719	22,896	37,492	21,880	842,618
1925	9	9	51,565	25,448	52,823	25,802	1,204,024
1926			46,324	23,366	51,369	25,060	1,083,064

\* Production and sales were practically the same in 1916 and 1917, and no distinction was made between them.

What has been done with greensand, it is believed we can do also with the other conspicuous potash minerals, such as the potash feldspars of the New England, the Appalachian and the Rocky Mountain states, the potash shales of Georgia, the leucites of Wyoming and the alunites of Utah.

In addition to these great and inexhaustible resources are the saline deposits of Nebraska, Utah and California, which we are confident can in part be made to contribute their quota to the Nation's potash supplies.

The problem of obtaining potash from these American sources is one of by-products—other products obtained simultaneously to share the production costs—for without them, potash cannot be produced cheaply enough to compete in an unrestricted market.

*Potash Consumed in American Agriculture.*—American potash requirements are illustrated by the fact that during the year 1926, potash salts

were imported of a total of 815,714 tons valued at \$14,133,079. This exceeds by almost 1½ million dollars the importations for 1925, and by one million dollars, 1913, illustrating the growing importance of potash in American agriculture.

Importations of fertilizer salts into the United States for the period 1910 to 1926 inclusive, are shown below:

*A seventeen-year record of the potash fertilizer salts entered for consumption in the United States*

Fiscal year	Kainite	Manure salts	Muriate	Sulfate	Total
	long tons	long tons	long tons	long tons	long tons
1910	470,241	90,933	174,935	37,933	774,042
1911	586,474	169,105	191,324	47,441	994,844
1912	479,817	185,682	216,101	45,134	926,734
1913	466,184	172,556	199,854	42,877	881,471
1914	526,112	260,977	234,855	44,986	1,066,930
1915	79,124	66,411	102,882	21,705	270,122
1916	64	2,278	2,126	2,427	6,895
1917		324	606	656	1,586
1918		225	596	136	957
Calendar year					
1918			379	90	469
1919	51,274	43,511	20,716	1,263	116,764
1920	372,019	311,462	121,602	15,184	820,267
1921	69,076	38,648	71,109	11,124	189,957
1922	151,149	195,005	160,254	57,620	564,028
1923	167,708	269,394	135,497	63,741	636,340
1924	156,708	231,248	129,128	75,696	592,780
1925	182,828	384,232	161,028	68,952	797,040
1926	181,877	316,440	199,151	69,873	767,341

Potash in terms of  $K_2O$  imported into the United States for the years 1913 to 1926, inclusive, is shown in the table below:

*Potash, in terms of  $K_2O$ , imported into the United States  
for the years 1913 to 1926, inclusive  
(In short tons of  $K_2O$ )*

1913	270,720	1920	224,792
1914	207,089	1921	78,698
1915	48,867	1922	201,415
1916	7,885	1923	209,950
1917	8,100	1924	200,365
1918	7,957	1925	258,200
1919	39,619	1926	238,000

*The Texas Deposits.*—The Federal Government has undertaken the exploration by core drilling of that portion of western Texas and south-eastern New Mexico which the analysis of balings from numerous oil wells

has shown to be underlain by potash deposits. This comprehensive exploration it is believed will establish the presence and determine the location and extent of workable potash strata in this great saline deposit.

A single core obtained in Eddy County, New Mexico, affords the first quantitative data pertaining to the thickness and actual chemical composition of these strata. In essence, this core reveals: At a depth of 794 to 799 feet,  $3\frac{3}{4}$  feet of polyhalite, averaging 9 per cent  $K_2O$ ; at a depth of 986 to 96 feet, two layers of polyhalite,  $2\frac{2}{3}$  feet and  $2\frac{1}{2}$  feet, separated by  $2\frac{1}{3}$  feet of rock salt, the potash mineral averaging 14.6 per cent  $K_2O$ ; at a depth of 1267 to 1271 feet,  $3\frac{2}{3}$  feet of sylvinite, averaging 13.8 per cent  $K_2O$ ; at a depth of 1311 to 1314 feet, 3 feet of polyhalite, averaging 8.9 per cent  $K_2O$ ; at a depth of 1365 to 1367 feet, 2 feet of sylvinite, averaging 18.5 per cent  $K_2O$ —and at lower depths various other strata of polyhalite, sylvinite, kainite and langbeinite.

### SUMMARY

The world-wide search for potash deposits to increase the world's supplies of agricultural potash, has resulted in the establishment of the existence of potash reserves enormous in extent and by nature quite inexhaustible.

These deposits may be classified into two groups—those containing potash in a form naturally soluble in water (saline), deeply buried, subterranean deposits, and those containing potash in combinations that are not soluble in water, potash minerals, surface deposits.

Compiled on this basis, the following tabulation gives an approximation of the present known and surveyed potash deposits of the world.

*The World's estimates potash reserves*  
(In tons  $K_2O$ )

Country	Saline	Mineral
	tons	tons
Africa	1,500,000	
France	300,000,000	
Germany	2,000,000,000	
Italy		8,000,000,000
Poland	4,000,000	
Russia	1,000,000,000	
Spain	268,000,000	
United States	22,000,000	2,500,000,000
Total	3,595,500,000	10,500,000,000

This compilation is incomplete in that it fails to take into consideration many minor deposits and sources of potash. For example, it does not

consider the potash feldspar nor the potash-bearing industrial wastes. Among the potash minerals here included are only those of definite segregations and for which processes are already evolved for the extraction of potash therefrom.

From the foregoing it is obvious that the world's supply of potash is adequate in point of quantity for the world's agriculture, not only as constituted at present but as it will be developed in the future. Certainly, intensive agriculture will be the prevailing type practiced, a situation that will be forced by increasing populations and organized agricultural economics. Intensive fertilization will be a *sine qua non* of intensive agriculture. At that time potash use will be many fold that of the present. But the controlling factor will not be the quantities of potash available, but the price at which it is obtainable. What the world's agriculture demands and will always demand is cheap potash. What it is at present concerned with is not so much increased reserves as lower prices. Present potash producers are energetically directing their efforts toward reduced production costs—by consolidations, centralization of plants, enlargement of production units and the development of side-products to share manufacturing costs. What can be done it seems safe to say, will be done, for the problem rests in the hands of the chemist and his ingenuity is well recognized. But a most important item of cost lies outside of his province to correct, and that is transportation. When he has gotten his product into as concentrated a form as possible, he has done his utmost to reduce that charge. Here is the most potent factor operating to restrict more liberal potash use, transportation costs.

German potash reaches the Atlantic seaboard bearing a charge of not less than \$5 per ton. Transportation from the seaboard inland to the fields where applied increases this charge at a rapid rate, so that a cost is soon reached where the use of potash in liberal applications to moderate priced crops is no longer regarded as economically feasible. It would be difficult to imagine potash from any other foreign source reaching our fields at a price lower than that exacted by the efficient and well organized German producers. The American farmer has all the potash he wants at present prices. The German-French combine are anxious to sell him many fold his present consumption. The American farmers' requirement obviously is not increased supplies but lowered prices and the only way to obtain the latter is lower transportation costs through shortening the distances that potash must be transported. This can only be done through developing potash industries close to the agricultural areas where the potash is to be applied. This constitutes the present potash problem—the problem which many nations are now trying to solve through the development of domestic potash industries.

In America this problem is more acute than in any other country, principally on account of its great area and the size and importance of its

agricultural industries. It may happen that the development here of a single large potash industry will be of little avail if that industry be not situated close to the areas of greatest potash demand. By way of illustration, transportation charges from the German mines to the agricultural southeast are less than those from our present greatest potash unit situated in California. The problem here accordingly is the development of potash industries on the basis of those raw materials which are found most advantageously situated with respect to agricultural areas. These are industrial wastes and potash minerals requiring chemical processing. Conditions as they exist impose difficulties which must be met and overcome. A fundamental principle with respect to the recovery of agricultural potash from these sources is that the potash to be cheap must be produced as a by-product or together with by-products to share the manufacturing costs. Researches are accordingly being directed toward the elaborations of as many as possible of the ingredients of these raw materials into merchantable products.

The elements that characterize the American potash problem apply with certain modifications to the potash problem of the world. World agriculture demands for its logical development the more liberal use of fertilizers. Unless increased prices are to be obtained for agricultural products, liberal use of fertilizers must in general be preceded by reductions in the costs of fertilizing elements. The world's supplies of agricultural potash, however great, to be adequate for future requirements must be cheap. The margin of profit resulting from its use must be more easily and more certainly discerned. The conservation of soil fertility is of fundamental importance. It is of such importance that it should be kept before every nation as a most pressing conservation measure. To the individual farmer, however, it is of importance more in the abstract and yields to the more concrete and more immediate problem of increased yields per unit of land and labor employed and per dollar expended. Increase in quantity and quality of crop is what interests him most. On this he bases his decision to use or not to use potash fertilizers. Costs therefore become of prime importance. And the reduction in costs of agricultural potash becomes the main element for consideration in the world's potash problem.

# ATMOSPHERIC NITROGEN FIXATION

F. A. ERNST

*United States Department of Agriculture, U. S. A.*

## INTRODUCTION

Although the atmosphere which completely envelopes the world is 80 per cent nitrogen, the first attempt to utilize this inexhaustible supply was made only 25 years ago. For centuries the ships of the inorganic nitrogen consuming countries have sailed to far off India and later, since 1830, to Chile, to bring back the natural nitrates of these lands to meet the ever increasing nitrogen demand.

Over every square kilometer of the earth's surface is some 700,000 metric tons of nitrogen, and yet it was not until after the warning sounded in 1898, of the danger of ultimate starvation of the peoples of the earth because of the early exhaustion of the natural nitrate deposits, that serious consideration was given to the possibilities of the industrial fixation of this atmospheric nitrogen.

The inorganic nitrogen consumption of the world in 1902, the year of the starting into operation of the first plant for the commercial fixation of atmospheric nitrogen, was 310,000 metric tons, composed of 212,000 as Chile nitrate, and 98,000 tons as a by-product of coke oven operation.

Today, of an annual consumption of nearly 1,500,000 tons, or five times the consumption of 25 years ago, less than half is of nitrogen as Chile nitrate and by-product ammonia. In fact, only 30 per cent of this increase is being supplied by these materials, while 70 per cent of the increase is made up of the nitrogen of the atmosphere fixed in utilizable form.

As the first attempt to fix atmospheric nitrogen on a really industrial scale was made on American soil, it is fitting that a résumé of the progress of this important industry be given on its twenty-fifth anniversary before this international delegation, holding its first Soils Science Congress on American soil.

## RÉSUMÉ OF METHODS OF FIXATION

In 1902 the Atmospheric Products Co., an American corporation, capitalized at \$1,000,000, erected a plant for the fixation of the nitrogen of the atmosphere according to patents granted Bradley and Lovejoy. This method of fixation is known as the arc process, since fixation is accomplished in the intense heat of the electric arc. The only raw

material necessary is air, which is approximately four-fifths nitrogen and one-fifth oxygen. This nitrogen is fixed with the oxygen to form nitric oxide, which when further oxidized and absorbed in water forms nitric acid. The power consumption, however, is very large.

Although the chemical energy absorbed in fixing one metric ton of nitrogen is only 0.205 kilowatt year, the total electrical energy consumption is about 7.3 kilowatt years, or 64,000 kilowatt hours.

The plant of the Atmospheric Products Company closed down in 1904, after nearly 2 years of attempted operation. Nitrogen had been fixed but the yield per unit of power employed proved insufficient, while the equipment proved to be too fragile for economical operation.

The new industry was kept alive, however, by the work in Norway during this time, of Professor Christian Birkeland and Engineer Samuel Eyde. These technologists, profiting by the knowledge of the difficulties encountered in the American attempt, developed a furnace which proved to be both a technical and economical success. The first Birkeland and Eyde plant was started into operation at Ankerlokken, near Oslo, in the fall of 1903, with a furnace employing 112 kilowatts. From this small beginning the present large Norwegian nitrate industry has grown, and to Norway goes the distinction of establishing this new industry on a permanent basis.

Of a present installed arc capacity of 42,000 metric tons of nitrogen per year, 35,000 tons is installed and operated in Norway by the Norsk Hydro-Elektrisk Kvaelfstofaktieselskab (Norwegian Hydro-electric Nitrogen Co.). This 35,000 ton capacity is divided between two plants, one at Notodden, of a capacity of 7,000 tons of nitrogen per year, and utilizing 50,000 kilowatts of electrical energy. The other at Rjukan has an annual fixation capacity of 28,000 tons of nitrogen with a total power consumption of 200,000 kilowatts.

Other arc furnaces which have been developed and put into commercial operation are those of Schönherr, Pauling, Guye, Moscicki and Wielgolaski. A large number of Schönherr furnaces are in operation at the Norwegian plants. The installations of the other furnaces are relatively small, totaling 7,000 tons of nitrogen per year at plants located in Germany, France, Austria and the United States.

Because of the large electrical power consumption per unit of nitrogen fixed, the adaptability of the arc process was limited to the few locations of very low-priced electrical energy.

During the days of the industrial development of the arc process, two eminent German scientists, Frank and Caro, in investigating a process for the production of alkali cyanides for gold extraction, worked up a method for the fixation of atmospheric nitrogen in the form of calcium cyanamide. The power consumption of the cyanamide process is less than one-fourth that of the arc process and resulted in a rather rapid growth of the industry.



The first plant erected was that at Westeregeln near Magdeburg, Germany, in 1905, with a capacity of 800 tons of cyanamide per year. Although this plant was not successful and was abandoned in 1908, a second and successful plant was started into operation, 1905, at Piano d'Orta, Italy, with a capacity of 4,000 tons of cyanamide per year. The successful operation of this plant led to the starting into operation of a plant at Bramberg, Prussia, in 1908, and in Trostberg, Bavaria, in 1909. There are now cyanamide plants in thirteen different countries. By 1910 the production of nitrogen fixed by the cyanamide process almost equalled that by the arc process, while by 1911, had passed it, and by 1912 it had almost doubled the production by the latter process.

The growth of the industry was very rapid, reaching its peak in 1918, with a rated installed capacity of 325,000 metric tons in 36 plants. Today there are 28 plants in existence, with a rated capacity of 285,000 tons of nitrogen per year. A single plant, the U. S. Nitrate Plant No. 2, at Muscle Shoals, Alabama, has a capacity as great as the total arc process capacity. Although the cyanamide plant capacity is approximately seven times the installed capacity of the arc process, the total energy consumption for such a production is less than twice the consumption necessary for operation of the installed arc capacity.

The third method of fixation which has attained industrial success is also the work of German genius. This is the direct synthetic ammonia process. While it had long been believed that it should be possible to effect a direct union of nitrogen and hydrogen to form ammonia, the difficulties attending such a union had baffled many investigators. A series of papers by Prof. Fritz Haber in 1905 and 1906, however, followed in 1909, by a patent granted this investigator, covering a process whereby nitrogen and hydrogen in the presence of a reaction promoter combined directly to form ammonia aroused commercial interest. By 1910 the Badische Anilin und Soda Fabrik had become so interested in the commercial possibilities of the process that the rights to Haber's patent were secured. Under the skillful guidance of such technicians as Dr. Bosch the inventor's experimental data were successfully worked into an industrial plant, which produced during the year 1913, the first year of operation, 6180 metric tons of nitrogen fixed as ammonia.

This first year's production was a third as much as the total 1913 production by the arc process. By 1915 direct synthetic ammonia process production exceeded that by the arc process, and at the present time such production is more than double the combined production of both the arc and cyanamide processes. Operation at this time is at the rate of over 600,000 metric tons of nitrogen per year, while there is 100,000 tons additional capacity under construction.

This process is not necessarily dependent upon electrical energy at all. The gas mixture ready for synthesis may be heated electrically or other-

wise, while the motive power for compression and circulation may be by any means. The total energy supplied may be considered the equivalent of 4,000 kilowatt hours per ton of nitrogen fixed, or but approximately one-fourth that of the cyanamide process.

One of the larger problems of the process is the production of the large quantities of hydrogen necessary. This hydrogen may be produced by the electrical decomposition of water, in which case the total electrical energy requirement per ton of nitrogen fixed is about 16,000 kilowatt hours. Only 18 per cent of the nitrogen fixed by this process is fixed with electrolytic hydrogen however, 15 per cent represents hydrogen from the electrolysis of water, and 3 per cent from other electrochemical processes as a by-product. The necessary hydrogen for the remaining 82 per cent of the nitrogen fixed by this process is secured through coal, 70 per cent from water-gas process, and 12 per cent from by-product coke oven gas.

There are now 55 plants of this process either operating or building, in fifteen different countries. Thirteen countries of Europe, Japan and the United States comprise these 15 countries, while the Cia Electrico de Adulos Quimicos Alkalis (Hydro-Electric Chemical and Alkali Co.) has announced its intention of erecting a direct synthetic ammonia plant in Brazil, South America, for fertilizer production.

There are several methods of operation of the direct synthetic ammonia process, all achieving the same result, the direct fixation of nitrogen with hydrogen to form ammonia. There are commercially four well-known methods, all known by the names of the inventors, who represent three different countries—Germany, France and Italy. The well-known Haber process is, as has been stated, the result of the work of Professor Haber of Germany. France has produced Claude, whose method is well-known, while two sons of Italy, Fauser and Dr. Luigi Casale, whose sudden death in February of this year, we who were fortunate enough to know him, all mourn, are internationally known through the methods of operation bearing their names.

### CAPACITY OF PRODUCTION

Progress in the industrial fixation of atmospheric nitrogen has favored, as has been shown, that process of lower power consumption. The result of this has been that although the total annual power consumption for the fixation of nitrogen today is at the rate of 7,000,000,000 kilowatt hours as compared with less than 1,000,000 the first year of production, the average consumption for the three processes combined is 10,000 kilowatt hours per ton of nitrogen fixed, as compared with 70,000 kilowatt hours 25 years ago.

The present combined capacity of the three processes is about 1,000,000 tons of nitrogen per year. Additional plants are being built and plans

are being made for still greater expansion. Approximately 85 per cent of this nitrogen is fed to the soil as fertilizers. It may be interesting, then, for such a gathering as this, to consider the possible further expansion of this industry.

Lipman<sup>1</sup> has estimated that the annual loss of nitrogen from the soil in the United States alone is 8,000,000 metric tons. Of this amount 4,900,000 tons is returned through manures, legumes, atmospheric precipitation, non-symbiotic bacteria and commercial fertilizers, leaving a net annual loss of 3,100,000 tons. As commercial fertilizers constitute only 200,000 tons of this nitrogen returned to the soil, it can be seen that the present rate of expansion of the atmospheric nitrogen fixation industry can continue for years before production will equal this loss.

How close actual production will approach this loss and the speed of such approach it appears is dependent upon such an economic consideration as price. If the cost to the consumer is low enough it is reasonable to assume that the demand will increase; doubtless an increased demand will be met with increased production, while it may be taken for granted that the supply of the raw material atmospheric nitrogen will be sufficient to meet any increased production no matter how great it may be.

Future progress or expansion in the fixation of atmospheric nitrogen then will be largely dependent upon the price at which the nitrogen of the atmosphere can be fixed in the various forms necessary for its use.

### SOURCES OF CONSUMPTION

There are three general sources of consumption of inorganic nitrogen: (1) explosives for both war and peace purposes, (2) the chemical industry including refrigeration, and (3) fertilizer.

Although in the United States alone there is consumed annually 500,000,000 pounds of explosives for such peaceful purposes as mining, quarrying, road-building and clearing land for cultivation, and although about 30,000,000 pounds of ammonia is used annually for refrigerating purposes, while large additional quantities of fixed nitrogen are consumed in the manufacture of photographic films, artificial leather, imitation ivory, dyes, household ammonia, and in the manufacture of soda-ash, yet approximately 65 per cent of the total is consumed in fertilizer or fertilizer materials. In the other countries of the world the percentage consumed in fertilizers is even larger so that perhaps 80 per cent of the world consumption of inorganic nitrogen is in the form of fertilizer materials.

With the advent of fixed atmospheric nitrogen at fertilizer prices has come a gradual change in fertilizer demands. The more immediate products of the fixation processes are largely rather concentrated materials.

<sup>1</sup> Jour. Amer. Soc. of Agronomy, Vol. II, No. 9, December 1919.

In this country mixed fertilizers of bulky materials have been used. As an example, there is the 2-8-2 mixture containing only 2 per cent of ammonia, 8 per cent of  $P_2O_5$  and 2 per cent of  $K_2O$ , or a total of 12 per cent of plant food. The remaining 88 per cent is what is known as carrier and filler. The carrier consists of such materials as, for instance, sulfuric acid in the formation of ammonium sulfate, while the filler is an inert material added to the mixture of fertilizer materials to make a ton of mixed fertilizer of 12 per cent plant food content. This practice of dilution has grown up largely through the use of mechanical drills for feeding the fertilizer to the soil. A change to more concentrated fertilizers is, however, taking place with the development of the newer compounds.

In Europe where fertilizers are applied largely by hand, the practice has been of the simpler method of applying each material separately. For instance the nitrogen is applied at one time in the form of sulfate of ammonia, cyanamide, Chile nitrate, while perhaps later an application of acid phosphate is made to supply the phosphorus and perhaps still later muriate of potash. Here too, however, the development of new compounds is working a change and the change is toward a single fertilizer containing the three plant foods such as do our mixed fertilizers but perhaps in a more concentrated form.

It is evident that the various commercial fertilizer consuming countries are approaching the same result in the progress of fertilizer practice, and that is towards concentrated materials containing more than a single plant food.

The cause of this working toward a single goal is undoubtedly fixed atmospheric nitrogen, and more definitely the direct synthetic ammonia process, the immediate product of which, ammonia, is not usable as a fertilizer material.

If the fixation of nitrogen with hydrogen from water gas is considered, it can be seen that urea is a very logical final product.

When steam is blown through a bed of glowing coal or coke a gas (water gas) is given off composed almost entirely of hydrogen, carbon dioxide and carbon monoxide. By treating this gas with steam in the presence of a catalyst or reaction promoter, the carbon monoxide is further oxidized to carbon dioxide by the oxygen of the steam liberating hydrogen. There remains a mixture of hydrogen and carbon dioxide. These two gases are relatively easily separated when the hydrogen is available for the fixation of nitrogen to ammonia. There remain, then, the two gases, ammonia and carbon dioxide. If these two gases are brought together under pressure in an autoclave maintained at the proper thermal conditions, the resulting product urea is a material of about 46 per cent nitrogen content and available as a fertilizer material.

Another concentrated material, and one which it appears at this time is to become of wide usage, is ammonium phosphate. This may be pro-

duced in two forms, the mono- and diammonium phosphate. The former contains about 75 per cent plant food (14 per cent  $\text{NH}_3$  and 61 per cent  $\text{P}_2\text{O}_5$ ), while the latter contains 78 per cent plant food (25 per cent  $\text{NH}_3$  and 53 per cent  $\text{P}_2\text{O}_5$ ). Ammonium phosphate is produced by neutralizing phosphoric acid with ammonia.

A combination of processes well adapted to the production of ammonium phosphate is that of the direct synthetic ammonia process with the Liljenroth process.

The Liljenroth process consists in producing elemental phosphorus from phosphate rock, silica and coke, in an electric furnace, condensing the phosphorus vapor and then passing a mixture of the phosphorus vapor with excess steam through a catalyst bed, producing phosphoric acid and hydrogen. Here the hydrogen is available for ammonia production, whence the phosphoric acid can be neutralized with the ammonia to ammonium phosphate. This method is in operation commercially in Germany. The elemental phosphorus produced at the cyanamide plant at Piesteritz is processed at the Badische Leunawerke, where it is neutralized with synthetic ammonia.

Of course, ammonium phosphate may be manufactured from materials produced by other methods such as the autoclaving of cyanamide to ammonia and the wet method of treating phosphate rock with sulfuric acid to form phosphoric acid. Such a method is in operation in this country at Warners, N. J., where the American Cyanamide Company is producing a material called "Ammophos." The disposal of this product is largely through export to the Orient.

The oxidation of ammonia to nitric acid has afforded a source of nitrate nitrogen. Ammonia oxidation, first operated on a large scale during the war for munitions purposes, is now undergoing rapid development as a source of fertilizer nitrate nitrogen. Ammonia gas mixed with either air or oxygen is passed over a highly heated catalyst material to form nitric oxide, which when further oxidized and absorbed in water forms nitric acid. Nitric acid may be a conversion product of either the direct synthetic ammonia or cyanamide processes for nitrogen-fixation, while it is the immediate fixation product of the arc process.

Nitric acid neutralized with ammonia produces a salt, ammonium nitrate, of 35.6 per cent nitrogen content. Because of its hygroscopicity, however, it, as such, has not proved popular. A large quantity of a double salt of ammonium-sulfate ammonium-nitrate (Leuna saltpeter) produced by the Badische Anilin und Soda Fabrik, is being used in Germany and other countries and has met with general approval. The Badische is now exploiting a material, nitrophoska, for both home and foreign markets. This material contains all three plant foods and the mixture for use in this country contains 16 per cent  $\text{NH}_3$  equivalent nitrogen, 32 per cent  $\text{P}_2\text{O}_5$ , and 16 per cent  $\text{K}_2\text{O}$ .

Other nitrogenous fertilizer materials are under investigation and like those previously mentioned are all of the more concentrated type, containing more than one of the general plant foods. It appears that concentrated fertilizers of this type are quite the result of synthetic nitrogenous materials and that the fixation of atmospheric nitrogen is resulting in an international method of soil fertilization.

## BORON AS A TOXIC CONSTITUENT OF ARID SOILS

W. P. KELLEY AND S. M. BROWN

*University of California, U. S. A.*

Several years ago Kellerman suggested that the pronounced toxicity of certain soils in the arid region of Western America might be due to boron. He pointed out that there are areas of varying size ranging from a few square yards to hundreds of acres in extent, scattered here and there over a considerable portion of California, Nevada and Arizona, on which there is scarcely a vestige of natural vegetation. In some of these soils there is not a high concentration of any of the ordinary salts that are common to arid regions.

It is well known that deposits of borax occur in several places on the Mojave Desert and in Death Valley, California. Boron is also a notable constituent of various brines and lakes in widely scattered localities in California and Nevada. It does not appear to be so well known, however, that boron is a minor constituent of the ground water, mineral springs and surface streams in many places, nor that noncommercial quantities of borax also occur in many localities in these states. The mineral colemanite, which is calcium borate, occurs in relatively large quantities in certain mountainous regions of California. Since, as will be pointed out a moment later, boron is extremely toxic to plants, the solubility of calcium borate is sufficiently high to lend some importance to its occurrence in water sheds from which irrigation supplies are drawn.

Several years ago Conner called attention to the fact that boron is extremely toxic to agricultural plants. His conclusions have since been abundantly confirmed by various other investigators. In connection with interesting experiments on the function of boron in plant growth, Dr. Brenchley, Miss Warrington, Dr. Summers, Collings and others have shown that a small amount of boron is an absolute necessity for the normal growth of many species. On the other hand, they noted that injury results if the concentration of boron exceeds a very low level.

For several years certain lemon groves in California have presented a peculiar appearance. The margins of the leaves and the tissue between the veins become chlorotic especially in the fall and winter. As the injury advances the margins of the leaves become brown, followed by excessive shedding of the leaves. The appearance is distinctly different from that produced by the ordinary alkali salts or other known causes. The soils in question are quite free from the ordinary alkali salts and

many of the affected trees were entirely normal for many years after the orchards were planted.

Recently we have discovered that boron is the chief cause of the difficulty. In certain places small amounts of soluble boron compounds are native to the soil, but the chief source of the boron is the irrigation supply. In our investigations we have produced definite injury to the lemon tree within a period of six months by the monthly application of an irrigation water that contained only 5 p.p.m. of boron. The symptoms thus produced were identical with those which occur in several large lemon orchards. Similar symptoms have resulted from the application of natural irrigation supplies which contain not more than 2 p.p.m. of boron. There is in fact considerable evidence that the regular application of irrigation water containing even less than 1 p.p.m. of boron may produce definite injury to citrus trees in the course of a few years' time. The injury is not confined to the lemon, although this species is especially sensitive to boron injury. The orange and grapefruit may also be affected.

The cultivated areas where boron is now known to be a toxic factor are scattered over several counties, ranging from Ventura on the north to Mexico on the south. We have found definite instances of boron injury in the Imperial Valley of California and near Yuma in Arizona. We have also obtained some evidence that boron may be related to the sudden collapse of certain deciduous fruit trees in the Sacramento Valley as far north as Tehama County, California.

A peculiar feature is found in the fact that the lemon has the power to absorb relatively large amounts of boron from extremely dilute solutions and to concentrate the same in the leaves. For example, more than 1000 p.p.m. of boron has occasionally been found in the dry matter of the affected leaves, and 400 to 500 p.p.m. is a common occurrence. These concentrations are always associated with visible evidences of injury. Since boron tends to accumulate in the vegetative portions of the plant, analysis of the leaf affords a valuable means of diagnosis.

Although the investigation is only begun, it is already evident that the extreme toxicity of boron necessitates a consideration of its possible occurrence as a toxic factor in alkali soils generally, and especially in those regions where boron minerals are known to occur. It is not improbable, as Kellerman pointed out, that the extreme sterility of many areas dotted here and there over Western America may be due in part at least to the occurrence of small amounts of boron. The economic importance of this problem is large. Some of the most important irrigation supplies of Southern California contain boron and the soils in question are among the most valuable in the state.



# CELLULOSE AS A SOURCE OF "HUMUS" IN THE SOIL<sup>1</sup>

S. A. WAKSMAN

*New Jersey Agricultural Experiment Station, U. S. A.*

Chemists and agriculturists have tacitly assumed in the past that celluloses, among the constituents of plant materials which are constantly added to soil, play a predominant rôle in contributing to the formation and accumulation of soil organic matter or "humus," (Detmer, Duclaux, Czapek). This assumption was not based upon any experimental evidence, since the very nature of this soil "humus" was only little understood; it was merely a logical conclusion of two facts: 1. celluloses were known to form the largest single chemical group of constituents of natural organic matter of plant origin, and 2. the addition of these organic substances to the soil results in the formation of black substances which were termed collectively "humus."

The chemical nature of this "humus" was a matter of considerable discussion and a subject of numerous speculations. Some investigators believed that this "humus" represents a single compound ("humic acid") or a group of closely related compounds ("humic," "hymetome-lanic," "fulvic," "crenic," and other acids, as well as "humin," "ulmin," and other ill-defined substances, all of which designate only certain preparations and not definite chemical substances). Other investigators succeeded in separating from the soil a number of definite chemical substances, leaving some doubt, however, as to the actual existence of these substances in the soil itself. It became a matter of common practice in tests and scientific discussion to refer to all dark-colored substances, whether formed biologically or produced by the action of concentrated acids upon carbohydrates, as "humus" compounds.

During the last few years, however, some concentrated attacks upon this subject have been made and definite light has been thrown if not upon the nature, at least upon the origin of the black colored substances in the soil.

Hoppe-Seyler was the first to demonstrate that the decomposition of pure celluloses by microorganisms does not give rise to black colored substances or "humus." Unfortunately, Hoppe-Seyler allowed the decomposition of the celluloses to take place either under anaerobic condi-

<sup>1</sup> Paper of the Journal Series, N. J. Agricultural Experiment Station, Department of Soil Chemistry and Bacteriology.

tions, or by the inoculation of his medium only with anaerobic organisms. His results could, therefore, not be interpreted in terms which would apply to normal soils. Bermi also expressed the idea that cellulose does not contribute to the formation of humus, either by natural or by artificial processes.

Of the numerous contributions following the work of Hoppe-Seyler on the subject of the rôle of celluloses in the formation of dark colored substances in the soil, it is importance to note the work of Trussov, who definitely demonstrated that no "humic acids" are formed in the decomposition of celluloses. He found that "humic acids" are formed from proteins, or rather certain protein derivatives, especially those having a pyrrhol or phenol group which give rise to oxyquinones and pyrrhols, and also from lignins, tannins and fats. The work of Fischer, Schrauth and others definitely established the rôle of lignins in the formation of "humus" in the soil. According to these investigators, when plant substances are added to the soil, the sugars, starches, celluloses, hemicelluloses and proteins are rapidly decomposed, while the lignins and to a less extent the fats and waxes are allowed to accumulate. A detailed review of this subject is given elsewhere. Attention will only be called here to the recent work of the author, which points definitely to the rôle of celluloses in the formation of "humus" in the soil. This work will tend to explain a number of various discrepancies observed by different investigators.

Various contributions to the subject of digestion of natural organic materials point to the great resistance of lignins to digestion both by animals, by lower invertebrates and by most microorganisms. König and Murdfield, as well as Bach found that in the animal digestive system celluloses are decomposed very readily by the intestinal bacteria, while the lignins are attacked only to a limited extent. The high carbon content of manure is explained by the relative increase in the lignin content. Dore and Miller have established that in the digestion of wood by *Toredo naavalis*, most of the celluloses and hemicelluloses are decomposed in the digestive system, while the lignins are hardly acted upon. These results as well as the work of Beckmann and Liesche, Pringsheim and Fuchs, and others support the lignin hypothesis, namely that the accumulation of "humus" in soil is due to the great resistance of lignins to decomposition. Waksman and Tenney also reported that in the decomposition of cereal straw by filamentous fungi and the complex soil flora, the lignins are decomposed to a much less extent than the other plant constituents. The removal of the lignins even hastens the decomposition of the celluloses by soil organisms. However, it must be recognized that even lignins are decomposed by soil organisms, as shown by Pringsheim and Fuchs, Schrader and Falk, but the process is a very slow one.

On the other hand, some of the very recent workers (Donath and Lissner, Potonie, Marcusson, and others) still persist in their idea that

cellulose plays an important part in the formation of peat and coal. Their contention is based largely upon the presence of celluloses in peat and coal, especially upon the rapidity with which oxy-celluloses may change into "humus"-like substances.

However, neither group of investigators attempt to explain the origin of the nitrogen in the "humus." This nitrogen is usually present in the soil organic matter, or the so-called "humus," in very definite concentrations (namely, about 3 per cent nitrogen in the purified "humus" or "humic acids"). More so, investigators have overlooked the fact entirely that soil organic matter always contains carbon and nitrogen in a very definite ratio ( $C:N=8-12:1$ ).

But, in view of the fact that neither did the presence of nitrogen fit in with any particular formulae for the various "humic acids," nor could the nature of its origin in the soil "humus" be interpreted, the only way to explain its presence in soil organic matter was to consider it as an impurity of the "humic acids." However, all attempts to purify the "humic acid" did not result in the complete removal of the nitrogen, although it seemed to be somewhat more soluble in boiling acids and in alkalies than the non-nitrogenous part of the soil organic matter.

The lack of a sufficient understanding of these phenomena was largely due to the fact that it was commonly assumed that the microorganisms decompose the pentosans and celluloses, proteins and sugars, without leaving any residue. Even as late as 1926, we have a suggestion to free lignin from celluloses by utilizing the cellulose decomposing power of fungi and bacteria. As if the fungi and bacteria are just catalysts which assist in carrying out a reaction without increasing or diminishing in the amount of the catalytic agent. The important fact that these catalysts are living organisms which grow and multiply, use energy, nitrogen and minerals and synthesize new cell substance is usually not taken into consideration. The author has definitely established the fact that a constant ratio exists between the amount of cellulose decomposed and nitrogen required, in the decomposition of cellulose by fungi, this ratio being about 30 to 1. A similar ratio is found to hold true also in the case of the decomposition of cellulose by bacteria. In other words, celluloses will not be decomposed if available nitrogen is absent or present in insufficient amounts. The use of celluloses as sources of energy by fungi and bacteria will *always be accompanied* by a parallel assimilation of nitrogen (and phosphorus) and by a *synthesis of microbial protoplasm*.

It is to the activities of the microorganisms that decompose the celluloses that we must look for the source of nitrogen in the soil "humus," as well as to the source of a large part of the "humus" itself.

Of the 3 different groups of microorganisms which are largely concerned in the decomposition of cellulose in nature, the fungi are most active in acid soils (below pH 6.0) and the bacteria and actinomyces are

largely active in faintly acid, neutral and alkaline soils, although fungi are able to decompose celluloses even under these conditions.

TABLE 1.—Decomposition of pure cellulose by different organisms

Name of organism	Period of incubation	Cellulose decomposed
	days	per cent
<i>Aspergillus fumigatus</i>	21	92.1
<i>Penicillium</i> sp. (No. 134)	21	87.7
<i>Trichoderma kőningi</i>	21	93.5
<i>Fusarium</i> 115	21	76.6
<i>Humicola</i> sp.	30	84.4
<i>Mucor racemosus</i>	Do	Do
<i>Cunninghamella elegans</i>	Do	Do
<i>Zygorhynchus mőlleri</i>	Do	Do
<i>Actinomyces violaceus ruber</i>	21	6.8
Do	42	18.0
<i>Act. cellulosa</i>	42	44.4
<i>Actinomyces</i> 292	Do	29.1
<i>Bacterium fimi</i>	42	29.0
Do	60	31.9
Bacterium No. 5 (orange)	30	37.7
<i>Spirochaeta</i> (impure culture) in solution	30	29.3
Do	30	25.4
Do	30	36.6
<i>Spirochaeta</i> in soil		59.4

Table 1 shows the rapidity with which different organisms are capable of decomposing celluloses. The following method was used for determining the ability of the various organisms to decompose celluloses. One g. portions of cellulose, in the form of well ground filter paper are added to 100 g. of soil or sand, to which 20 cc. of a nutrient solution (10 g.  $(\text{NH}_4)_2\text{HPO}_4$ , 3.0 g.  $\text{K}_2\text{HPO}_4$ , 2.0 g.  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 1.0 g.  $\text{NaCl}$ , 0.02 g.  $\text{FeSO}_4$ , 1000 cc. distilled water) and 1 g.  $\text{CaCO}_3$  are also added. The flasks containing the medium were sterilized, inoculated with pure cultures of the respective organisms and incubated at 28° C. for 21 to 60 days. At the end of the incubation period, the remaining cellulose was determined by the method of Charpentier, while the residual ammonia is determined by the method of extraction with potassium chloride solution and distilling with  $\text{MgO}$ .

Whatever the chemistry of the processes of cellulose decomposition by microorganisms, whether they are decomposed largely to organic acids and alcohols, as in the case of some bacteria, or entirely to  $\text{CO}_2$  and  $\text{H}_2\text{O}$ , as in the case of most fungi one phenomenon is of outstanding importance, namely the synthesis of microbial protoplasm. This can be measured quantitatively in three ways: 1. the disappearance of the inorganic

(ammonia or nitrate) nitrogen, 2. the formation of organic nitrogen compounds, 3. the synthesis of carbon in the microbial cells.

TABLE 2.—Cellulose decomposition and nitrogen assimilation by microorganisms

Name of organisms	Nature of medium	Incubation	Nitrogen used up	Cellulose decomposed	Cellulose Nitrogen
		days	mg.	mg.	
<i>Aspergillus fumigatus</i>	Sand	21	29.4	920.9	31.3
<i>Penicillium</i> 134	Do	Do	26.5	877.2	33.1
<i>Fusarium</i> 115	Do	Do	23.0	766.0	33.3
<i>Trichoderma kőningi</i>	Do	Do	29.2	935.0	32.0
<i>Humicola</i>	Do	42	16.3	844	51.7
<i>Actinomyces cellulosa</i>			12.8	444.0	34.7
<i>Spirochaeta cytophaga</i> No. 1 <sup>a</sup>	Solution	30	11.0	293	26.6
Do No. 2	Do	Do	9.0	254	28.2
Do No. 2	Do	60 <sup>b</sup>	8.1	360	44.4
Orange-colored bacterium	Do	30	15.0	377	25.1
<i>Spirochaeta cytophaga</i> No. 1	Sand	42	10.0	594	59.4
Do No. 2	Do	42	6.0	306	51.0
Do	Soil	Do	11.4	570	50.0

<sup>a</sup> The *Spirochaeta* culture was accompanied by a small rod-shaped bacterium.

<sup>b</sup> Insufficient amount of available nitrogen, all of which is used up.

The results reported in Table 2 show that there is a definite correlation between the amount of cellulose decomposed and the microbial protoplasm synthesized in the case of fungi, actinomyces and bacteria. This ratio varies with the nature of the medium, length of incubation period, and environmental conditions, as well as with the nature of the organism and presence of other organisms.

The large quantities of nitrogen assimilated by the microorganisms point to the considerable quantities of cell substance built up by the various organisms in the process of cellulose decomposition. The fact that a more or less definite ratio exists between the cellulose decomposed and nitrogen assimilated points to the existence of a definite ratio between the cellulose decomposed by at least all the aerobic cellulose decomposing organisms and the cell substance synthesized by these organisms. As a matter of fact this cell substance has actually been measured, as shown elsewhere. Just how the cell substance is built up, whether by decomposing the cellulose to acetaldehyde, as shown by Neuberg and Kohn, then forming amino acids or by other processes still remains to be determined.

A large part of the carbon of the cellulose decomposed by the aerobic fungi and bacteria is given off as carbon dioxide. It is of interest to compare the amounts of carbon of the cellulose decomposed, carbon of the carbon dioxide given off into the atmosphere and the carbon of the cell

substance synthesized. The carbon content of cellulose is obtained by multiplying the amount of cellulose decomposed by 44.4 per cent. The carbon content of the gases given off is obtained by multiplying the results for the CO<sub>2</sub> evolution by 27.5 per cent. The carbon of the cell substance synthesized is obtained by multiplying the nitrogen assimilated by 9, for fungi (fungi containing 45 per cent carbon and about 5 per cent nitrogen) and by 6 for bacteria (bacterial cells containing about 45–48 per cent of carbon and about 8 to 10 per cent of nitrogen) Table 3.

TABLE 3.—Carbon balance in cellulose decomposition

Name of organism	Age of culture	Medium	Cellulose decomposed	CO <sub>2</sub> produced	Cell substance synthesized (calculated)
	days		mg. of carbon	mg. of carbon	mg. of carbon
<i>Trichoderma</i>	17	Solution	124.8	71.4	54.9
Do	38	Do	326.8	182.8	138.3
Do	14	Sand	408.9	125.6	266.6
Do	21	Soil	400.0	209.5	195.3
<i>Penicillium</i>	18	Sand	288.1	141.8	155.6
Do	21	Soil	255.2	162.2	201.6
<i>Spirochaeta</i>	30	Sand	199.8	98.6	79.7
Do	30	Soil	254.4	158.7	84.6
<i>Bacterium</i>	28	Solution	167.2		87.6

The results brought out in Table 3 point definitely to the fact that the fungi, as represented by the *Trichoderma* and *Penicillium* do not leave any intermediary products in the decomposition of celluloses (see also Fig. 1). All the carbon of the cellulose decomposed is changed completely to carbon dioxide and synthesized cell substance. In the case of the *Spirochaeta*, the sum of the carbon of the gas and cell substance synthesized is less than the carbon of the cellulose decomposed, due to the fact that this organism forms certain organic acids, which are left in solution and that a considerable amount of a gum-like polysaccharide is also synthesized (bacterial slime).

The results of these investigations as well as of numerous others reported elsewhere bring out clearly the fact that the organisms, which decompose the celluloses, pentosans and the other constituents of the natural organic materials are capable of synthesizing considerable quantities of cell substance. In the case of some organisms, as much as 30 to 40 per cent of the carbon of the cellulose decomposed can be stored in the form of microbial protoplasm. As shown elsewhere, this protoplasm is further decomposed by various bacteria, actinomyces, protozoa and invertebrate animals; a part of the carbon is changed again to CO<sub>2</sub> and a part of the

nitrogen to ammonia. (This ammonia is either again assimilated by the cellulose-decomposing fungi and bacteria, if free cellulose or pentosan are available, or it is changed to nitrate and made available for plant growth). However a part of this synthesized organic matter resists further decomposition and forms a very important source of soil organic matter or soil "humus." This synthesized "humus" is soluble in alkali solutions, giving the typical dark color of soil "humus," and is rich in nitrogen. To bring out these facts more definitely, the results of the following experiment are reported here.

Five hundred g. portions of pure quartz sand were placed in a series of porcelain pots. Twenty-five g. portions of pure filter paper and 120 cc. of a nutrient solution containing 800 mg. of nitrogen in the form of ammonium phosphate  $((\text{NH}_4)_2 \text{HPO}_4)$  or ammonium sulfate  $((\text{NH}_4)_2 \text{SO}_4)$  as well as a small amount (3 g.) of  $\text{K}_2\text{HPO}_4$ , some  $\text{MgSO}_4$ ,  $\text{KCl}$  and  $\text{FeCl}_3$  were added to each pot. The pots were inoculated with a dilute suspension of fresh garden soil, covered with glass plates and placed in the incubator at 25 to 28° C. At the end of about 6 weeks, all the paper, having previously become covered with a growth of various fungi and bacteria, became decomposed. Fresh 25 g. portions of paper were added and sufficient distilled water to keep the mixture at optimum moisture for aerobic organisms (60 to 70 per cent of moisture holding capacity). Additional nitrogen was added only when a test had shown that the ammonia was being all used up. Repeated additions of celluloses were continued every 3 or 4 weeks until at the end of 1 year, just 275 g. of paper, equivalent to 253 g. of pure cellulose, and 2800 mg. of combined nitrogen was added to each pot. Practically all the paper had disappeared; instead there was left a dark-brown mass consisting of the particles of white sand surrounded with organic matter, largely in a colloidal condition.

A careful analysis has shown that there was left in each pot 71.795 g. of organic matter, of which 25.495 g. was cellulose. In other words, the decomposition of 227.505 g. of cellulose ( $253 - 25.495 = 227.505$ ) resulted in the accumulation, as a result of the synthesizing agencies of the microorganisms, of 46.3 g. ( $71.795 - 25.495 = 46.3$ ) of new organic matter. The ratio between the organic matter synthesized and the cellulose decomposed is

$$\frac{46.3 \times 100}{227.505} = 20.35 \text{ per cent.}$$

Of the 2.800 g. of nitrogen added to the sand medium 2.688 g. was found at the end of the experiment, the slight difference being probably due to experimental errors. Of this nitrogen 1.180 g. was in the form of ammonia. The microorganisms that brought about the decomposition of 227 g. of cellulose, have thus assimilated 1.508 g. ( $2.688 - 1.180$ ) of nitrogen which has been changed into complex microbial proteins and

other nitrogen complexes. The nitrogen content of the synthesized residual organic matter is thus found to be

$$\frac{1.508 \times 100}{46.3} \text{ or } 3.26 \text{ per cent}$$

The synthesized organic matter, namely the 46.3 g., consisted of the following fractions:

Soluble in cold water = 9.045 g. of 19.53 per cent

Soluble in 4 per cent NaOH solution = 19.005 g. or 41.05 per cent

Soluble in 2 per cent H<sub>2</sub>SO<sub>4</sub> solution = 8.900 g. of 19.22 per cent

Organic matter, insoluble in water, dilute alkalies and dilute acids = 9.350 g. or 20.20 per cent

The portion soluble in cold water contained 199 mg. of nitrogen in a combined organic form.

The alkali soluble portion contained 988 mg. of nitrogen. In other words, while the water-soluble portion of the organic matter contained only about 2.2 per cent of nitrogen, the alkali soluble portion contained 5.2 per cent of nitrogen. While only 41 per cent of the organic matter is soluble in 4 per cent NaOH solution (heating 30 minutes at 15 lb. pressure) but not in water, 65.6 per cent of the nitrogen is thus made soluble.

When the alkaline solution is treated with hydrochloric acid, a heavy precipitate is formed. On boiling the mixture, then filtering and analyzing it was found that the precipitate weighed 1.465 g., or 3.16 per cent of the total organic matter; its nitrogen content was 57.8 mg. or 3.95 per cent, and its ash content about 1 per cent. In other words, as a result of decomposition of 227 g. of cellulose, the microorganisms of the soil synthesized 46.3 g. of "humus," similar in color and behavior to the soil "humus." This "humus" contained 1.465 g. of "humic acid," a portion soluble in alkalies and precipitated by acids. This "humic acid" contained 3.95 per cent nitrogen. When a portion of the sand containing the synthesized material was treated with concentrated hydrochloric and sulfuric acid, for lignin determination, about 1500 mg. of lignin was actually found per each pot of the material. When it is recalled that the "humic acids" obtained from the soil contain about 3 per cent nitrogen and when it is recalled that the procedure of obtaining them was the same, the source of "humus" and "humic acid" become evident.

One can very readily understand why Schalbe and Eckenstam found that the lignin from wood attacked by fungi was soluble 64.2 per cent in alkali solutions, while lignin from natural wood was only 8.4 per cent soluble. This assumption as well as the ideas of Wehmer that as a result of the activities of the fungi "humin" substances and lignic acids are formed are quite correct.



The sand-organic-matter mixture was taken out from the pots, well mixed and divided into 2 portions. One (1) was placed back in pots (on the original basis of 500 g. of sand in each pot) and to another, (2), 1 per cent of  $\text{CaCO}_3$  was added. The mixtures were further incubated, in the presence of optimum moisture for 4 months. At the end of that period they were again analyzed, now in greater detail, the mixture not receiving any  $\text{CaCO}_3$  being referred to as 1 and the mixture receiving  $\text{Ca}_2\text{CO}_3$  as 2.

*Composition of sand-organic-matter mixture*

pH of mixture	1 6.0	2 7.4
	grams	grams
Total organic matter left (by ignition)	58.430	42.438
Cellulose content of residual organic matter	18.912	8.891
Total synthesized organic matter	39.518	33.547
Nitrogen content of organic matter	2.835	2.726
Ammonia nitrogen present	1.709	1.479
Nitrate nitrogen present	0	220
Organic nitrogen content of synthesized organic matter	1.126	1.027
Per cent of nitrogen of organic matter	2.85 per cent	3.06 per cent

The synthesized organic matter diminished in the 4 months of incubation from 46.3 to 39.518 g., in the absence of  $\text{CaCO}_3$  and to 33.547 g., in the presence of  $\text{CaCO}_3$ . The more rapid decomposition of the organic matter in the presence of  $\text{CaCO}_3$  is due to a greater activity of the bacteria and actinomyces. The mixture not receiving any  $\text{CaCO}_3$  contained, by the plate method, 1,500,000 fungi and 5,400,000 bacteria per gram (very few actinomyces). The mixture receiving  $\text{CaCO}_3$  contained 120,000 fungi and 176,000,000 bacteria (15 to 30 per cent of which were actinomyces colonies).

Among the constituents of the synthesized organic matter which were first to decompose, the nitrogenous substances seemed to occupy a prominent place, as seen both by the diminution of the nitrogen content of the residual synthesized organic matter and by the rapid increase of the available nitrogen in the form of ammonia and nitrates. This points definitely to the fact that in soil processes the synthesis of organic matter by microorganisms is sooner or later followed by processes of decomposition, whereby certain constituents are rapidly decomposed and the nitrogen is again changed from organic nitrogen into soluble nitrogen, largely ammonia, which is oxidized to nitrate in the presence of  $\text{CaCO}_3$ . However, a considerable part of the synthesized organic matter persists in the soil and is more or less resistant to decomposition.

A detailed analysis of the remaining synthesized organic matter gave the following results:

*Analysis of remaining synthesized organic matter*

	1	2
Fats and waxes, soluble in ether . . . . .	941 mg.	475 mg.
Per cent of total synthesized organic matter	2.38 per cent	1.41 per cent
Soluble in cold water, 24 hour extraction	11.942 g.	7.841 g.
Per cent of total synthesized organic matter	30.23 per cent	23.37 per cent
Nitrogen content of organic matter (-ammonia and nitrate nitrogen)	267 mg.	206 mg.
Soluble in hot water, boiling for 30 minutes	2.720 g.	4.488 g.
Per cent of total organic matter	6.88 per cent	13.37 per cent
Nitrogen content of hot water soluble organic matter (-ammonia nitrogen)	109 mg.	186 mg.
Organic matter soluble in 5 per cent NaOH (60 minutes at 15 lb. pressure)	10.048 g.	7.986 g.
Per cent of total organic matter . . . . .	25.44 per cent	23.80 per cent
Nitrogen content of soluble part	596.8 mg.	529.3 mg.
Synthesized "humic acid" (Part of alkaline solution precipitated with HCl, at boiling temperature)	2.096 g.	2.178 g.
Per cent of total residual synthesized organic matter . . . . .	4.8 per cent	4.9 per cent
Nitrogen content of the "humic acid"	100.4 mg.	106.1 mg.
Per cent of nitrogen in the synthesized so-called "humic acid" . . . . .	5.75 per cent	5.79 per cent
Organic matter soluble in 2 per cent H <sub>2</sub> SO <sub>4</sub> solution	1.722 g.	2.046 g.
Per cent of total organic matter	4.36 per cent	6.13 per cent
Organic matter insoluble in dilute alkalies and acids (chitins, etc.)	11.238 g.	9.351 g.
Per cent residual synthesized organic matter (so-called "humins")	28.43 per cent	28.87 per cent
Nitrogen content of the insoluble organic matter	59. mg.	86.1 mg.

A careful analysis of the results shows that the synthesized organic matter contains all the various fractions commonly found in the soil organic matter, although in an unequal proportion, namely the water-soluble portion of the synthesized organic matter is considerably higher than of the organic matter commonly found in the soil. This is a result of the fact that in the soil the synthesized organic matter has already undergone considerable decomposition, as a result of which the water-soluble portion is the first to disappear; this leads also to an increase in the amount of the alkali-soluble portion. Between 50 to 75 per cent of the soil organic matter is soluble in 4 per cent NaOH solution (on heating under pressure), while only 25 per cent of the synthesized organic matter

is soluble in alkalis. This fraction, however, is most resistant to decomposition, and increases in the soil, with the advance of decomposition. The part of the synthesized organic matter which is insoluble in cold and hot water and in hot 4 per cent NaOH solution and 2 per cent  $H_2SO_4$  solution, which comprises the chitins and other constituents of microbial cell substance, is equivalent to the so-called "humins" and "ulmins" commonly recorded in the soil.

The most interesting part is the nitrogen content of the synthesized organic matter, which is about 3 per cent, while the nitrogen content of the "humic acids" found in the synthesized organic matter is a trifle less than 5 per cent. This tends to explain several important soil phenomena: The standard ratio of carbon to nitrogen in the soil which tends to be 10 or 12 to 1. This ratio is found to hold true of the alkali-soluble part of the synthesized organic matter, which is precipitated by acid, namely the part most resistant to decomposition. This fraction has 50 to 55 per cent carbon and 4 to 5 per cent nitrogen.

Organic matter in soils, where decomposition processes are not very active, as acid forest soils, water-logged forest and peat soils, etc., has a much wider carbon-nitrogen ratio, frequently 20:1 and even 30:1. This is due to the great abundance of lignins (with 63 per cent carbon and no nitrogen) and other constituents of natural organic materials, which resist decomposition under those conditions and which have a high carbon and a low nitrogen content. Two other phenomena bear out this point: the low nitrogen content of the "humic acid" of these soils and the high "humin" content, or that part of the organic matter which is insoluble in alkalis, at low pressures and temperatures. The "humic acid" on the synthesized organic matter is high in nitrogen and is readily soluble in alkalis.

## SUMMARY

The results presented in this paper can be briefly summarized as follows:

Celluloses are decomposed in nature largely by the agency of four groups of organisms: (a) Fungi, which are most active in acid and well aerated soils; these fungi include a great many genera found among the Ascomycetes, Basidiomycetes and Fungi Imperfecti. (b) Aerobic bacteria, which are most active in neutral, alkaline and faintly acid soils. The soil has to be well aerated and contain free bases ( $CaCO_3$ , etc.). They include various *Spitochaeta*, Vibrio's and spore and non-spore forming bacteria. (c) Actionmyces, active under conditions favorable to aerobic bacteria, especially when the moisture content is below the optimum. (d) Anaerobic bacteria, active in water-logged soils and under anaerobic conditions in general.

The decomposition of celluloses is always accompanied by the syn-

thesis of considerable cell substance by the microorganisms which are active in the process. Since the cell substance synthesized by the organisms contains a definite amount of nitrogen and since those organisms that decompose the celluloses are unable to use atmospheric nitrogen, the decomposition of celluloses will lead to the transformation of inorganic nitrogen into complex organic nitrogen compounds as constituents of the microbial cell substance. It has been established that there is a definite ratio between the cellulose decomposed and the nitrogen used by the organisms of the synthesis of cell substance, this ratio being about 30 to 1 for the fungi and aerobic bacteria. In the presence of other microorganisms, which do not decompose the celluloses, but attack the synthesized cell substance, a part of the nitrogen is again liberated in an inorganic form. This may be again assimilated, in the presence of an excess of cellulose, thus leading to a widening of the cellulose-nitrogen ratio.'

The synthesized cell substance may amount to 20 to 30 per cent of the cellulose decomposed. When the synthesized cell substance in its turn undergoes decomposition, certain constituents, which are similar in their chemical reactions to lignins and to "humic acids," but which are readily soluble in alkalies and which contain 3 to 5 per cent nitrogen remain. These are more resistant to decomposition than the other cell constituents. While the total synthesized organic matter contributes to the soil "humus" with all its characteristic properties, the alkali-soluble constituents of the synthesized organic matter contribute to the so-called "humic acids" of the soil:

These results tend further to confirm the theory proposed previously that soil organic matter or soil "humus" is made up of two groups of substances of different origin: (a) constituents of plant material, which resist decomposition, like the lignins, cutins, etc., (b) constituents of the synthesized cell substance, which resist decomposition; this cell substance having originated by the agencies of microorganisms that used the celluloses, pentosans, starches, sugars and proteins as sources of energy.

This mass of organic matter or "humus" accumulates only at low temperatures, under anaerobic conditions and under acid conditions. Under aerobic conditions, at high temperatures, in the presence of free bases, this "humus," including the lignins, fats and waxes and synthesized cell substances decompose by certain specific organisms, as will be shown later.

#### LITERATURE CITED

- (1) Bach, M. *Landw. Vers. Sta.* 1926. 104: 245.
- (2) Beckmann, E., and Liescher, E. *Biochem. Ztschr.* 1923. 139: 491.
- (3) Benni, S. *Ztschr. Naturwiss.* 1896. 69: 145.
- (4) Charpentier, C. A. G. 1921. *Diss. Helsingfors.*

- (5) Czapek, F. *Biochemis der Pflanzen*. 1905. 1: 226.
- (6) Detmer, W. *Bodenkunde*. 1874.
- (7) Donath and Lissner, *Brennstoffchem.* 1921. 2: 37.
- (8) Dore, W. H., and Miller, R. C. 1923. *Univ. Calif. Publ. Zool.* 22: 383.
- (9) Duclaux, E. *Chimie biologique*. p. 219, 805.
- (10) Falck, R.
- (11) Fischer, Fr. et al *Brennstoff*. 1921-1922. *Chem.* 2:37 (1921); 3: 65, 161; (1922); *Die Naturwiss.* 9: 958 (1921).
- (12) Heukelekian, H., and Waksman, S. A. 1925. *Jour. Biol. Chem.* 66: 323.
- (13) Hoppe-Seyler, F. *Ztschr. physiol. Chem.* 1889, 13: 66.
- (14) König, J., and Murdfield, R. 1914. *Landw. Vers. Sta.* 65: 557.
- (15) Lieser, Th. *Cellulosechemie*, 1926, 7: 156.
- (16) Marcusson, J. Z. *Angew. Chem.* 1921-1925. 35: 339 (1925); *Ber. deut. Chem. Gesell.* 54; 542 (1921).
- (17) Neuberg, C., and Cohn, R. 1923. *Biochem. Ztschr.* 139: 527.
- (18) Oden, S. *Kolloidchem. Beihefte*, 1919. 11: 75.
- (19) Potomie, *Braunkohle*, 1922. 21: 365.
- (20) Pringsheim, H., and Fuchs, W. 1923. *Ber. deut. Chem. Ges.*, 56: 2095.
- (21) Schrader, H., 1922-1923. *Chem. Centrbl.* 4: 1044 (1922); 1649 (1923).
- (22) Schrauth, W. *Brennstoffchem.* (1923). 4: 161.
- (23) Schwalbe, C. G., and Ekenstam, A. 1917-1927. *Cellulosechemie.*, 1927. 48: 13.
- (24) Trussov, A. G. 1917. *Contrib. to the study of Russian soils.* Petrograd, 26-27: 1-210.
- (25) Waksman, S. A., 1924. *Jour. Agr. Sci [England]*. 14: 555.
- (26) ———. *Soil Sci.*, 1926, 22: 123.
- (27) ———. *Die Naturwiss.* (1927).
- (28) ———, and Skinner, C. E. *Jour. Bact.*
- (29) ———, and Tenney, F. G., *Soil Sci.*, 1926, 22: 395.
- (30) Wehmer, C. *Ber. deut. chem. Gesell.* 1915, 48, 1: 130.\*

## PAPERS NOT SUBMITTED

The following is a list of papers presented before the various sections of Commission IV but which were not submitted for publication. The page reference given is that of the Abstracts of the Proceedings of the First International Congress of Soil Science, unless otherwise indicated:

### **BEURTEILUNG DER BÖDEN AUF GRUND DER DERZEITIGEN METHODEN ZUR BESTIMMUNG DES REAKTIONS- BEZW. KALKZUSTANDES DER BÖDEN**

O. LEMMERMANN

*Landwirtschaftliche Hochschule, Berlin-Dahlem, Deutschland*

Page 4

### **ÜBER DIE SCHWANKUNGEN DES KALKGEHALTS IM ROTBUCHENLAUB AUF VERSCHIEDENEM STANDORT**

G. KRAUSS

*Tharandt-Dresden, Deutschland*

Page 5

### **AN IMPROVED METHOD FOR THE STUDY OF PLANT NUTRIENTS IN SAND CULTURES**

A. G. McCALL

*University of Maryland, U. S. A.*

Page 22

### **SOME EFFECTS OF FERTILIZING AND LIMING OF THE ACID PEAT SOILS OF NORTH CAROLINA**

L. G. WILLIS

*North Carolina Agricultural College, U. S. A.*

Page 25

**STUDIES ON THE ABSORPTION OF ELEMENTS BY CERTAIN  
AGRONOMIC PLANTS AS AFFECTED BY THE ABSENCE  
OF VARIOUS SALT ELEMENTS IN THE CULTURE  
MEDIA (BOTH AQUEOUS AND SOLID) BUT  
SUBSEQUENT TO EXPOSURE FOR VARI-  
OUS GROWTH PERIODS TO COM-  
PLETE CULTURE MEDIA**

W. F. GERICKE

*University of California, U. S. A.*

Page 31

**THE INFLUENCE OF NITROGEN, PHOSPHATE AND  
POTASH ON THE GROWTH, QUALITY AND  
MATURITY OF COTTON**

J. J. SKINNER

*United States Department of Agriculture, U. S. A.*

Page 34

**ON CERTAIN EXPERIMENTS FOR THE UTILIZATION OF  
NATURAL ALUMINUM PHOSPHATE**

Y. KIDA

*Imperial College of Agriculture and Forestry, Miyazaki, Japan*

Page 37

**THE EFFECT OF ACID PHOSPHATE UPON SOIL  
REACTION AND GROWTH OF ALFALFA**

M. C. SEWELL, W. L. LATSHAW AND E. L. TAGUE

*Kansas Agricultural Experiment Station, U. S. A.*

Page 45

**RELATIVE EFFECTIVENESS OF INORGANIC AND ORGANIC  
NITROGEN ON DIFFERENT SOIL TYPES**

B. E. BROWN

*United States Department of Agriculture, U. S. A.*

Page 47

**INFLUENCE OF THE CONTINUOUS CULTIVATION OF  
RICE ON SOIL FERTILITY**

T. IMASEKI

*Imperial Tokyo Sericultural College, Japan*

Page 63

**THE IMPORTANCE OF THE INTERRELATION OF SOIL  
TYPES IN AGRICULTURE**

A. R. WHITSON  
*University of Wisconsin, U. S. A.*

Page 70

**ON THE GENERAL OCCURRENCE OF MANGANESE  
COMPOUNDS IN PLANTS AND THEIR  
PHYSIOLOGICAL SIGNIFICANCE**

K. ASŌ  
*Tokyo Imperial University, Japan*

Page 77

**DIE WIRKUNG EINER ZWÖLFJAHRIGEN KALIDÜNGUNG  
AUF PFLANZE UND BÖDEN**

H. NIKLAS  
*Hochschule Weihenstephan, Deutschland*

Page 82

**GROWTH OF CERTAIN AGRONOMIC PLANTS IN MEDIA  
DEVOID OF VARIOUS SALT ELEMENTS AT DIFFER-  
ENT PERIODS OF GROWTH OF THE PLANTS  
AND SUBSEQUENT TO EXPOSURE TO  
COMPLETE CULTURE MEDIA**

W. F. GERICHKE  
*University of California, U. S. A.*

Page 92

**THE ORGANIZATION OF FIELD EXPERIMENTS WITH DIVERSE  
METHODS OF SOIL MANAGEMENT**

V. NOVÁK  
*Brno, Czechoslovakia*

**PLANT GROWTH AND PLANT YIELDS**

A. RIPPLE  
*University of Göttingen, Germany*



**INVESTIGATIONS IN THE FIELD OF APPLIED SOIL SCIENCE****A. A. JARILOV***Moscow, U. S. S. R.***FIELD INVESTIGATIONS OF THE APPLICATION  
OF SOIL SCIENCE TO AGRONOMY****G. P. KRAVKOV***Leningrad, U. S. S. R.***THE TRIANGLE SYSTEM FOR SOIL FERTILITY STUDIES****O. SCHREINER***United States Department of Agriculture, U. S. A.***METHODS OF QUANTITATIVE DETERMINATION OF NITROGENOUS  
PLANT NUTRIENTS (NITRATES AND AMMONIA)  
AND THEIR RELATION TO SOILS AND PLANTS****H. NIKLAS***Agriculture Institute, Weihenstephan, Germany*





**I. A. R. I. 75.**

IMPERIAL AGRICULTURAL RESEARCH  
INSTITUTE LIBRARY  
NEW DELHI.

Date of issue.	Date of issue.	Date of issue.
01.07.41		
17.7.41		
30.11.43		
21.12.44		
18.2.45		
2.12.47		
2.6.53		